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PROCEEDINGS

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SERIES B

CONTAINING PAPERS OF A BIOLOGICAL CHARACTER

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THE ROYAL SOCIETY.

SECTION B.—BIOLOGICAL SCIENCES.

The Chemical Action of Bacillus cloacæ *(Jordan) on Citric and Malic Acids in the Presence and Absence of Oxygen.*

By JAMES THOMPSON.

(Communicated by Arthur Harden, F.R.S. Received August 27,—Read November 14, 1912.)

(From the Biochemical Department, Lister Institute.)

The chemical action of *Bacillus cloacæ* on citric acid and on malic acid has not up to the present been investigated.

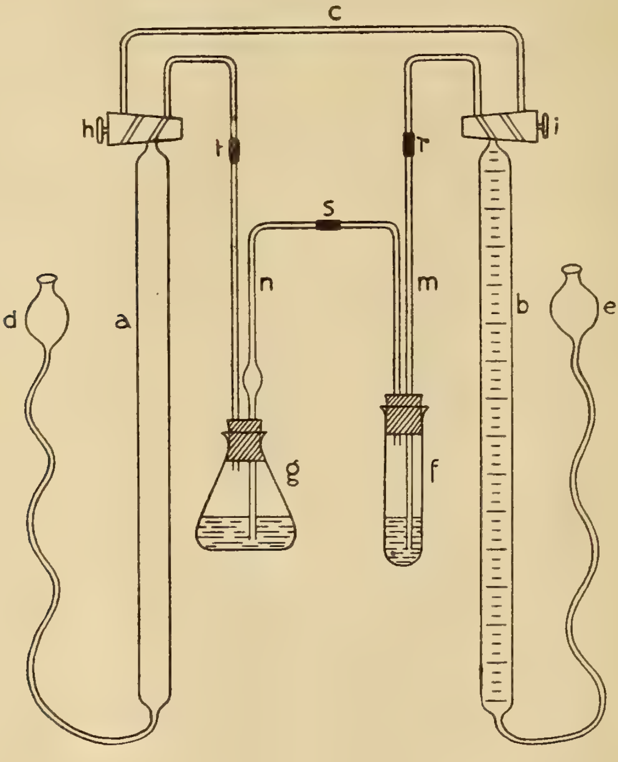
Since this organism is a facultative anaërobe, the experiments were undertaken with the object of ascertaining the effect of the presence of oxygen on the course of the fermentation produced. It was found, however, that the organism could not grow on ammonium malate in the absence of oxygen, so that the observations on this subject were limited to the case of citric acid.

Emmerling (1) has described the decomposition of malic acid by *B. lactis aërogenes* (Escherich), a very closely allied organism, and Bosworth and Prucha (2) have shown that, during the souring of milk, the citric acid present in fresh milk is converted by *B. lactis aërogenes* into acetic acid and carbon dioxide.

I. In investigating the chemical changes produced by the action of *B. cloacæ* on various media, the determination of the respiratory coefficient was first undertaken. For this purpose the apparatus described below, and shown diagrammatically in the annexed figure, was used.

Two burette tubes (*a*) and (*b*), fitted with two-way taps (*h*) and (*i*), are connected by a capillary tube (*c*). To each are attached by stout rubber tubing the pear-shaped receivers (*d*) and (*e*). The incubation tube (*f*), containing the medium under examination, and the flask (*g*), containing 50 c.c. of a 10 per cent. solution of caustic potash, are connected with the taps of the burettes as shown in the diagram, by stout rubber connections at (*t*) and (*s*). The internal capacity of the flask and incubation tube and connecting tubes between the taps (*h*) and (*i*) was determined. Deducting the volume of medium and caustic potash solution, the volume of air contained in them at the beginning of the experiment is known.

The burettes and receivers having been charged with mercury, the



incubation tube (*f*), containing 10 c.c. of the medium, previously sterilised and subsequently inoculated from a culture of *B. cloacæ* on the same medium, is connected to the burette (*b*) and the absorption flask (*g*). The tap (*i*) is removed, and, about 80 c.c. of air having been allowed to flow into the graduated burette, the tap is replaced in connection with the tube (*f*). By suitable manipulation of the receivers (*d*) and (*e*) and the two-way taps, the liquid in the tubes (*m*) and (*n*) is brought to the level of the liquid in (*f*) and (*g*). The volume of air, thus brought to atmospheric pressure, is then read off on the burette (*b*), the temperature and barometric pressure being carefully noted. The tap (*h*) is now opened to the flask (*g*), the receiver (*d*) being slightly lowered to create a partial vacuum in the burette tube (*a*). At intervals of 24 hours, the air in the burette (*b*) is swept through the apparatus into the burette (*a*) through the tube (*m*) by raising the receiver (*e*) and simultaneously lowering the receiver (*d*). Then, by reversing the taps, it is returned to the burette (*b*) through the tube (*c*).

By thrice repeating these manipulations, the carbon dioxide produced by the growth of the organism in the medium is removed from the tube (*f*), and absorbed by the caustic potash solution in (*g*). At the conclusion of the experiment, indicated by the non-diminution of the volume of air in (*b*), the flask (*g*) is disconnected, and the carbon dioxide determined by double titration with N/10 H_2SO_4, using phenolphthalein and methyl orange as indicators. The experiments were carried out at an approximate temperature of 37°.

It is essential that the burette taps be perfectly gas-tight. The form shown in the diagram was found to be the most satisfactory and the taps used were tested against a vacuum. As a lute, resin ointment proved to be the best for a temperature of 37°.

In making the readings, of which the initial and final are, of course, all important, it is essential to be quite sure that no marked alteration of temperature has taken place previous to observation. To obviate any error from this source, three readings were made in each case at intervals of 30 minutes, and their agreement showed that the experiment was being satisfactorily carried out.

To test the apparatus, a blank experiment was made, 10 c.c. of water being placed in the tube (*f*).

Internal capacity of apparatus = 176·8 c.c.

	Burette reading.	Temperature.	Barometer reading.	Volume of air at N.T.P.
	c.c.	°	mm.	c.c.
June 17, 12.30 P.M.	83·2	37·6	756·6	213·0
2.30 „	83·0	37·4	757·0	213·2
5.0 „	83·0	37·4	757·1	213·2
„ 19, 10.30 A.M.	82·8	37·8	758·7	213·0
The door of the hot room was allowed to remain open for 5 minutes. Fall in temperature 4·2°, rising quickly on again closing the door.				
June 19, 12.0 noon	80·2	35·4	758·7	214·3
3.0 P.M.	83·0	37·6	757·6	213·1

From this test it is seen that the apparatus described is quite satisfactory, provided that due precaution is taken that the burette readings are made while the apparatus is not subject to fluctuations of temperature.

Throughout the experiments detailed below, the air was circulated through the apparatus every 24 hours, readings being taken to check the course of the experiment. At the conclusion of each experiment, the residual gas in the apparatus was examined gasometrically for marsh gas and hydrogen, which were in each case found to be absent.

II. For the examination of the products resulting from the action of *B. cloacæ* on citric and malic acids, the general method described below was employed. The media were prepared from the acids by drying these to constant weight *in vacuo* over sulphuric acid, dissolving in distilled water, and, after neutralising with ammonia to convert them into their ammonium salts, adding sufficient normal sulphuric acid to give the finished product an acidity of +15. Acid potassium phosphate was added in the proportion of 0·1 grm. per litre of medium. The malic acid was present in the proportion of 6·7 grm. per litre, while, for the citric acid medium, the proportion used was 7 grm. of crystallised acid per litre, this quantity corresponding to 6·4 grm. of the anhydrous acid.

One litre of the medium was placed in a 2-litre flask, fitted with leading and delivery tubes plugged with cotton-wool. The delivery tube was connected outside the incubator with an absorption flask provided with a soda-lime tube, to allow of exit of the excess of air or nitrogen, and containing 200 c.c. of a 10 per cent. solution of caustic potash for the absorption of the carbon dioxide produced. After sterilisation, the medium was inoculated from a pure culture of *B. cloacæ* on agar, and a slow current of air, free from carbon dioxide, was bubbled through the apparatus during the course of growth. In the case of the experiments conducted in the absence of oxygen, the leading tube of the incubation flask was fitted with a three-way tap, by means of which a stream of nitrogen could be passed through the flask, after first displacing the air in the leading tube. The nitrogen was prepared by the action of ammonia solution on copper turnings, and passed through wash-bottles containing sulphuric acid, chromous chloride solution, and, finally, caustic potash. Growth in the incubation flask having ceased, which was found to be the case after about four weeks, the amount of carbon dioxide was determined by double titration of the caustic potash solution, and the contents of the incubation flask were examined as follows: The liquid was made up to 1 litre with distilled water, 500 c.c. were taken, made acid with 40 c.c. of normal sulphuric acid, and carefully distilled. The first 200 c.c. of distillate were collected, titrated with normal potash, using phenolphthalein paper as indicator, and redistilled. One hundred cubic centimetres of distillate were collected, and its specific gravity determined. In each experiment this was found to be equal to that of water, proving the non-production of any notable quantity of alcohol. The distillation of the original liquid was continued with steam, until titration of the distillate showed no further neutralisation of alkali. The combined titrated distillates were evaporated to low bulk, made up to 100 c.c. with distilled water, and examined for volatile acids. Formic acid, when present, was determined by heating 10 c.c. of the solution for two hours on the water-bath with 10 c.c of a 10 per cent. solution of sodium nitrite and 50 c.c. of a saturated solution of mercuric chloride, and weighing the precipitated mercurous chloride. From the weight found, the amount of formic acid was calculated.

The identity of the acids was established by determination of the molecular weight by means of the barium salt. The residue in the distilling flask was concentrated to 100 c.c., and completely extracted with ether in a continuous extraction apparatus. The ether was distilled off, and the residue dried at 100° and weighed. The identity of this residue with succinic acid was established by converting it into its barium salt. A weighed portion was heated for one hour on the water-bath with water and excess of barium carbonate. After filtration, the insoluble portion was well washed with hot water and the filtrate made up to 200 c.c.; 100 c.c. of this solution were evaporated to dryness, dried, and weighed. Conversion of this residual barium salt into barium sulphate showed it to consist of barium succinate, and proved that lactic acid is not produced by the growth of *B. cloacæ* on either malic or citric acid. Further proof that the residue was succinic acid was afforded by the determination of its melting point and by its reaction with ferric chloride.

The amount of residual acid, *i.e.* the acid not used up by the organism, was determined in the residue from which the succinic acid had been extracted with ether. This was made faintly alkaline with caustic potash, and evaporated to low bulk to get rid of ammonia. The residue was then taken up with water, exactly neutralised with acetic acid, and barium acetate added. Four volumes of 95 per cent. alcohol were then added, and the liquid allowed to stand overnight. In the case of the malic acid only a faint precipitation took place, showing that all but traces of the malic acid had been used up. In the case of citric acid, the precipitated barium salt was filtered off, washed with 63 per cent. alcohol, ignited, and finally weighed as barium sulphate, from which the amount of citric acid left was calculated.

In no case did the fermentation liquids give Vosges and Proskauer's reaction, thus proving that methylacetylcarbinol, which is produced by the action of *B. cloacæ* on mannitol and glucose, is not formed.

III. *Determination of the Respiratory Coefficient.*

The following tables show the results obtained with the apparatus previously described. In each case 10 c.c. of medium were employed. For Experiment 1 the figures are given in full, in order to show the course of the experiment; in the following experiments only the initial and final readings are given, though it is to be noted that they were similarly conducted. The day-by-day readings are essential, as the observation of any marked irregularity in the consumption of oxygen would point to some leak in the apparatus. The experiments were continued until the readings after an interval of 24 hours were the same.

Experiment 1.—Peptone Water.

	Internal capacity of apparatus.	Burette reading.	Temperature.	Barometer reading.	Volume of gas, corrected for N.T.P.
	c.c.	c.c.	°C.	mm.	c.c.
May 7	176·8	87·0	38·2	761·6	216·7
„ 8	„	87·2	37·8	759·1	216·7
„ 9	„	85·8	38·0	758·3	215·0
„ 11	„	80·2	36·8	762·4	213·4
„ 12	„	76·0	34·6	757·3	211·5
„ 13	„	76·4	36·2	756·1	209·3
„ 14	„	74·3	35·2	753·0	208·0
„ 15	„	71·2	35·6	755·7	205·7
„ 18	„	64·8	37·0	774·3	203·7
„ 19	„	65·8	37·4	771·0	203·0

Oxygen absorbed........... 216·7 − 203·0 = 13·7 c.c.

CO_2 produced .... 10·35 c.c.

Respiratory coefficient ... $\frac{10·35}{13·7} = 0·76$.

No. of experiment.	Substance.	Corrected volume of gas.		Oxygen absorbed.	CO_2 produced.	Respiratory coefficient, CO_2/O_2.
		Initial.	Final.			
		c.c.	c.c.	c.c.	c.c.	
2	Peptone ...	214·1	207·3	6·8	6·5	0·96
3	Malic acid	219·6	209·1	10·5	17·9	1·7
4	„	221·7	211·3	10·4	17·9	1·7
5	„	216·1	204·0	12·1	20·2	1·67
6	„	221·2	210·2	11·0	18·4	1·67
7	„	216·3	204·9	11·4	15·7	1·4
8	„	214·0	209·9	4·1	6·7	1·64
9	Citric acid...	220·3	214·6	5·7	13·4	2·35
10	„ ...	206·4	196·6	9·8	31·3	3·2

The mean result of the experiments gives for malic acid a respiratory coefficient of 1·63. This is in good agreement with the value found from a reaction proceeding as follows :—

$$3C_4H_6O_5 + 5O_2 = 2C_2H_4O_2 + 8CO_2 + 5H_2O,$$

which gives a respiratory coefficient of 8/5 = 1·6.

Experiments 9 and 10 were carried out with citric acid. The results are discussed later on (p. 9).

IV. *Chemical Action of* Bacillus cloacæ *on Malic Acid in the Presence of Oxygen.*

1. The only products of the action of *B. cloacæ* on malic acid in the presence of oxygen were found to be carbon dioxide, acetic acid, succinic acid, a small amount of a fatty substance, the nature of which was not determined, and traces of alcohol. The following tables give the results obtained. Table I gives the actual weight, on the malic acid used up, of the various substances produced, while Table II gives the number of carbon atoms per molecule of malic acid represented by each product :—

Table I.—Percentages.

	a.		*b.*	
	grms.	per cent.	grms.	per cent.
Carbon dioxide	3·40	50·8	3·25	48·5
Acetic acid	1·06	15·8	1·09	16·3
Succinic acid	2·02	30·2	2·36	35·2
Total	6·48	96·8	6·70	100·0
Malic acid consumed = 6·70 grms.				

Table II.—Carbon Atoms.

	a.	*b.*
Carbon dioxide	1·54	1·48
Acetic acid	0·71	0·73
Succinic acid	1·37	1·79
Total	3·62	4·00
Malic acid = $C_4H_6O_5$.		

In three further experiments, the carbon dioxide and acetic acid only were determined, the following results being obtained :—

	c.	*d.*	*e.*	
Carbon dioxide	2·86	2·36	2·39	grms.
Acetic acid	1·21	0·70	0·82	„

The molecular ratios $CO_2/C_2H_4O_2$ calculated by the formula $\frac{CO_2}{44} \times \frac{60}{C_2H_4O_2}$ are :—

Experiment.........	*a.*	*b.*	*c.*	*d.*	*e.*
Molecular ratio, $\frac{CO_2}{C_2H_4O_2}$	4·25	4·06	3·21	4·62	3·99

The action of *B. cloacæ* on malic acid in the presence of oxygen probably goes on in two ways : the one, an oxidation of the acid to carbon dioxide and acetic acid by atmospheric oxygen, and the other, an oxidation accompanied by reduction of a portion of the malic acid to succinic acid. The degree of access of the oxygen during fermentation will thus account for the difference in the proportions of succinic acid formed.

Assuming that growth in a sufficient supply of oxygen leads to complete oxidation of the malic acid to carbon dioxide and acetic acid, the following equation may be put forward as representing the changes which take place :—

$$3C_4H_6O_5 + 5O_2 = 2C_2H_4O_2 + 8CO_2 + 5H_2O.$$

This gives a ratio $CO_2/C_2H_4O_2$ of 4 and a respiratory coefficient of $8/5 = 1·6$, the latter being in good agreement with the results of the experiments previously described. In these experiments it is to be noted that a small quantity (10 c.c.) of medium is in contact throughout with a good supply of air, so that oxidation may be considered to be almost complete. If any oxidation were due to the oxygen derived from a second molecule of malic acid, the respiratory coefficient would be increased. On the other hand, oxidation of the acetic acid would decrease the coefficient. The numbers actually found were 1·67, 1·67, 1·7, 1·7, 1·64 and 1·4.

V. Contrary to the statement of Ritter (2), it was found that *B. cloacæ* would not grow on ammonium malate in the absence of oxygen.

VI. *Chemical Action of* B. cloacæ *on Citric Acid in the Presence of Oxygen.*

The substances produced from citric acid by *B. cloacæ* in the presence of oxygen were found to be the same as from malic acid. The amount of acetic acid produced is markedly greater in the former case, and the molecular ratio $CO_2/C_2H_4O_2$ for malic acid is four times as great as that for citric acid. Table III gives the results of an experiment showing the actual weights and percentages of the substances found, and the corresponding number of carbon atoms. In Table IV are given the results of three further experiments, in which the succinic acid and residual citric acid were not determined.

Table III.

	Grammes.	Percentages.	Carbon atoms.
Carbon dioxide	2·40	37·8	1·63
Acetic acid	3·36	53·0	3·39
Formic acid	0·07	1·2	0·05
Succinic acid	0·19	3·0	0·19
Residual citric acid	0·06		
Total	6·08	95·0	5·26

Citric acid used up = 6·4−0·05 = 6·35 grm.

Table IV.

	b.	*c.*	*d.*
	grm.	grm.	grm.
Carbon dioxide	3·48	2·58	2·73
Acetic acid	3·04	3·52	3·16
Formic acid	None	None	None

From these results the molecular ratios were calculated.

Experiment	*a.*	*b.*	*c.*	*d.*
Molecular ratio, $\frac{CO_2}{C_2H_4O_2}$	0·97	1·56	1·0	1·18

VII. *Action of* B. cloacæ *on Citric Acid in the Absence of Oxygen.*

In contrast with the growth of *B. cloacæ* in the presence of oxygen, that in absence of the latter results in the production of a small amount of formic acid. There is also an increased production of acetic acid, and the molecular ratio $CO_2/C_2H_4O_2$ diminishes markedly. Tables V and VI give the results of two experiments; in a third experiment (*c*), the carbon dioxide and acetic acid only were determined.

Table V.—Percentages.

	a.		*b.*	
	grm.	per cent.	grm.	per cent.
Carbon dioxide	1·64	28·8	1·84	31·2
Acetic acid	3·50	61·3	3·44	58·5
Formic acid	0·15	2·6	0·22	3·7
Succinic acid	0·17	3·0	0·03	0·5
Total	5·46	95·7	5·53	93·9
Citric acid used up	5·70	—	5·88	—

Table VI.—Carbon atoms.

	a.	*b.*
Carbon dioxide	1·26	1·36
Acetic acid	3·92	3·74
Formic acid	0·11	0·15
Succinic acid	0·19	0·03
Total	5·48	5·28
Citric acid = $C_6H_8O_7$.		

In a third experiment the carbon dioxide and acetic acid only were determined.

c.	Carbon dioxide	1·76 grms.
	Acetic acid	3·62 „

From these three experiments the following molecular ratios are obtained :—

Experiment	*a.*	*b.*	*c.*
Molecular ratio $\frac{CO_2}{C_2H_4O_2}$	0·64	0·73	0·66

Neglecting the small amounts of formic and succinic acids, this ratio corresponds with the following equation for the anaërobic decomposition of citric acid by *B. cloacæ*, in which the molecular ratio

$$CO_2/C_2H_4O_2 = \tfrac{6}{9} = 0{\cdot}67.$$

$$(1) \quad 4C_6H_8O_7 + 4H_2O = 9C_2H_4O_2 + 6CO_2.$$

The effect of the presence of oxygen is to increase the relative proportion of carbon dioxide with respect to the acetic acid. This may be due to the occurrence of reactions such as the following:—

$$(2)\quad 4C_6H_8O_7+2H_2O+2O_2 = 8C_2H_4O_2+8CO_2+2H_2O.$$

$$(3)\quad 4C_6H_8O_7+4O_2 = 7C_2H_4O_2+10CO_2+2H_2O.$$

These give the ratios shown in the following table, which also includes the numbers actually found:—

	Molecular ratio, $\frac{CO_2}{C_2H_4O_2}$.	Respiratory coefficient, $\frac{CO_2}{O_2}$.
Equation 1	0·67	
2	1·0	4·0
3	1·4	2·5
Found by experiment	0·97	
	1·0	
	1·56	
	1·18	
	—	2·35
	—	3·2

It is also possible that the decomposition takes place in the same way in the presence or absence of oxygen, according to equation (1), but that in presence of oxygen a portion of the acetic acid is subsequently oxidised.

Summary.

(*a*) Malic acid is not fermented by *B. cloacæ* in the absence of oxygen.

(*b*) Malic acid is decomposed by *B. cloacæ* in the presence of oxygen into carbon dioxide, acetic acid, and succinic acid, with traces of alcohol. The decomposition probably goes on in two ways; oxidation by atmospheric oxygen to carbon dioxide and acetic acid, and oxidation at the expense of another portion of the malic acid, which is thereby reduced to succinic acid.

With a good supply of air the respiratory coefficient CO_2/O_2 and the molecular ratio $CO_2/C_2H_4O_2$ found agree well with the values given by a reaction proceeding as follows:—

$$3C_4H_6O_5+5O_2 = 2C_2H_4O_2+8CO_2+5H_2O.$$

(*c*) In contradistinction to malic acid, citric acid is fermented by *B. cloacæ* in the absence of free oxygen. In addition to carbon dioxide, acetic, and succinic acids, the products resulting from aërobic fermentation, formic acid is produced, while there is an increased production of acetic acid.

The molecular ratio $CO_2/C_2H_4O_2$ found agrees with the value for the following equation:—

$$(1)\quad 4C_6H_8O_7+4H_2O = 9C_2H_4O_2+6CO_2.$$

(*d*) Citric acid is decomposed by *B. cloacæ* in the presence of oxygen into the same products as malic acid.

The values found for the respiratory coefficient and for the molecular ratio $CO_2/C_2H_4O_2$ are intermediate between those required for the following equations:—

$$(2)\quad 4C_6H_8O_7 + 4O_2 = 7C_2H_4O_2 + 10CO_2 + 2H_2O.$$

$$(3)\quad 4C_6H_8O_7 + 2H_2O + 2O_2 = 8C_2H_4O_2 + 8CO_2 + 2H_2O.$$

This is probably due to the difficulty of maintaining complete aëration of the medium during the experiment, the decomposition being therefore partly anaërobic and partly aërobic in character.

(*e*) It is possible that the decomposition of citric acid by *B. cloacæ* takes place in the same way in the presence or absence of oxygen, according to equation (1), but that in the presence of oxygen a variable portion of the acetic acid is subsequently oxidised.

(*f*) Methylacetylcarbinol, which is produced by the action of *B. cloacæ* on mannitol and glucose, is not formed in the fermentation of malic acid or of citric acid by this organism.

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The Origin and Destiny of Cholesterol in the Animal Organism. Part X.—*On the Excretion of Cholesterol by Man, when fed on Various Diets.*

By G. W. Ellis and J. A. Gardner.

(Communicated by Dr. A. D. Waller, F.R.S. Received September 3,—Read November 14, 1912.)

(From the Physiological Laboratory of the University of London, South Kensington.)

In earlier papers of this series we have shown that cholesterol is never excreted in the normal fæces of herbivorous animals such as horses, cattle, sheep, and rabbits. In the case of carnivora such as dogs and cats, provided the body weight remains constant, the cholesterol excreted in the fæces can be all accounted for by that naturally ingested with the food. Klein in his experiments also arrived at a similar conclusion. Evidence was also brought forward which rendered probable the view that, in herbivora, at any rate, cholesterol is a substance which is strictly conserved in the animal economy, that when the destruction of the red blood corpuscles and possibly other cells takes place in the liver, their cholesterol is excreted in the bile, and that the cholesterol of the bile is re-absorbed in the intestine along with the bile salts, finding its way into the blood stream to be used in cell anabolism; further, that any waste of cholesterol might be made up from that taken in with the food. This latter process would be limited in herbivorous animals by the fact that their normal food does not contain cholesterol, but isomeric substances such as phytosterol, which have to be converted into cholesterol before utilisation, and in carnivorous animals by the partial, or even complete, change of cholesterol into coprosterol which takes place under certain dietetic conditions. In man, under normal conditions, cholesterol is never excreted as such in the fæces, but always in the form of coprosterol. It seemed therefore desirable to estimate the amounts of coprosterol found in the fæces of man under various dietetic conditions. The opportunity of making such investigations was very kindly afforded us by Dr. R. H. A. Plimmer, who handed over to us the dried fæces collected during a series of experiments carried out in the Physiological Institute, University College, London, and published in the 'Journal of Physiology,' August 26, 1909, under the title of "A Metabolism Experiment, with Special Reference to the Origin of Uric Acid," by R. H. Aders Plimmer, Maxwell Dick, and Charles C. Lieb.

The subject of the experiment was a healthy man, aged 39. The three diets selected were chosen so that each yielded 110 grm. protein, 240 grm. carbohydrate, and 100 grm. fat per diem. The carbohydrate and fat constituents consisted of potato and butter, and the protein constituents of (1) beefsteak, (2) egg-white, or (3) herring-roe.

The experiment was commenced with an ordinary mixed diet for one week. After this the beefsteak diet was administered, and this was followed by the egg-white. After one week the subject unfortunately suffered from an attack of influenza, so that the experiment had to be discontinued for about 10 days, though analysis of urine was continued except for a period of four days. The egg-white diet was then taken for a period of 35 days. The herring-roe diet concluded the experiment, but this diet was taken only for three days owing to its bad effect on the patient. The fæces in this last period were not kept separate, but included with those obtained during the 35 days of egg-white diet. For further details as to the experiment the reader is referred to the original paper.

Cholesterol Contents of Various Diets.

The cholesterol content of the constituents of the various diets was determined by the digitonin method in the usual manner.

Diet I.—Total per diem :—

Beefsteak	500 grm.
Potato	800 „
Butter	100 „
Sugar....................	80 „

Meat, according to our own observations and those of others, may be taken as containing not less than 0·0685 per cent. of cholesterol—free and combined.

Butter, according to Magnus-Levy, contains 0·19 per cent. A sample of "best dairy butter" obtained from Harrods' Stores was found, by the digitonin method, to contain 0·1744 per cent. of cholesterol—free and combined.

The total cholesterol ingested in Diet I would, therefore, be at least $0{\cdot}3425 + 0{\cdot}1744 = 0{\cdot}5169$ grm. per diem.

Diet II.—Total per diem :—

Egg-white	800 grm.
Potato	800 „
Butter	130 „
Sugar....................	40 „

It was found that neither potato nor egg-white contains any trace of cholesterol. The total cholesterol ingested was, therefore, 0·227 grm. per diem.

Diet III.—Total per diem:—

Herring-roe	500 grm.
Potato	800 „
Butter	130 „
Sugar.....................	80 „

100 grm. of tinned herring-roe (soft) of the kind taken during the experiment was found to contain 0·595 per cent. of cholesterol—free and combined. The total cholesterol ingested was, therefore, $2{\cdot}975+0{\cdot}227 = 3{\cdot}202$ grm. per diem.

Treatment of the Fæces.

The fæces were supplied to us in a *dry*, finely powdered condition. Those from each series were thoroughly extracted with ether in a Soxhlet apparatus, and the fatty matter in the ethereal solution saponified with sodium ethylate. After separating the soap, the ethereal solution was thoroughly washed with water and evaporated to dryness. From the residues it was found possible to isolate a quantity of pure coprosterol by fractional crystallisation from alcohol. The mother-liquors were evaporated to dryness, and the residual coprosterol benzoated by means of benzoyl chloride in pyridine solution. The fæces from Series I, III, and IV, containing an excessive amount of oily impurity, rendered the isolation of pure coprosterol benzoate so difficult that the residues were further treated with digitonin, and in this way an amount of digitonin coprosteride was obtained. In order to be certain that the compound so formed was none other than the coprosteride, we recovered the coprosterol in combination by means of the xylene method.

Results of Analysis.

Series I.—In this series the subject was fed on an ordinary mixed diet for seven days. Five stools were passed, and yielded 167·7 grm. of dry material. The patient's weight was practically constant, varying from day to day from 75·8 to 76·2 kgrm.—average 75·99.

The fæces yielded on extraction 6·7445 grm. of unsaponifiable matter, from which 4·1669 grm. of coprosterol were obtained. This would correspond to a yield of 0·595 grm. per day. This daily output corresponds very closely to that found in the cases of one of us and another colleague on liberal diet from observations extending over a year. Fæces from a public latrine, however, yielded a smaller quantity.

Series II.—The subject was then fed for seven days on Diet I. His average weight was 75·3 kgrm., and varied on six of the days as follows: 76, 75·3, 75·3, 75, 75, 75·6. Four stools were passed, corresponding to

118·1 grm. of dry fæces. On extraction, this yielded 4·492 grm. of unsaponifiable matter, from which 3·3306 grm. of coprosterol were obtained. The balance sheet works out as follows:—

Total cholesterol (free and combined) ingested with food	3·6183	Total coprosterol excreted	3·3306
Intake per diem	0·5169	Output per diem	0·4758

Difference, 0·0411 of cholesterol per diem absorbed.

Series III.—Then followed seven days on Diet II. During this period the patient was sickening for an attack of influenza. His weight varied as follows: 75, 74·6, 74·2, 74·3, 74·3, 74·2—average 74·4 kgrm. The average loss in weight from the average of the previous period was thus 900 grm., or an actual loss during the week of 800 grm. Three stools were passed, containing 118 grm. of solid matter. This yielded 3·9605 grm. unsaponifiable matter, from which were obtained 2·6505 grm. of coprosterol. The balance sheet works out as follows:—

Total cholesterol ingested	1·587	Total coprosterol excreted	2·6505
Intake per diem	0·2267	Output per diem	0·3786

Excess of cholesterol excreted	1·0635
„ „ per diem	0·1519

If we reckon the loss in weight as due to fat and protein tissues the loss of cholesterol in this way would be from 0·45 to 0·8 grm., which would largely account for the excess excreted over intake.

Series IV.—During the next five days the patient suffered from influenza. Two meals of Diet II were taken and 35 grm. of dry fæces were obtained. The weights of the patient were as follows:—74·2, 72·6, 73·2, average 73·3. The fæces excreted during the first four days yielded 1·7638 grm. of unsaponifiable matter, from which 1·3109 grm. of coprosterol was obtained.

Total cholesterol ingested during period	0·4534	Total coprosterol excreted	1·3109

Excess of cholesterol excreted	0·8575
„ „ per diem	0·2144

The patient lost during illness 1·5 kgrm., so that about 1 grm. of cholesterol might be accounted for in this manner, and the excess of output over intake explained.

Series V.—During recovery from illness the patient was fed for six days on an ordinary mixed diet, after which the experiment was continued. He was fed for 33 days on Diet II, with addition of various salts, for one day on 100 grm. egg-white and 250 grm. boiled-out meat, for another day on 250 grm. of boiled-out meat, and for three days on a total of 400 grm. egg-white + 1250 grm. herring-roe.

The daily weights of the patient showed a steady decrease during this long period—from 73·8 to 70·7 kgrm.—a total loss of 3·1 kgrm. The total weight of dry fæces passed during the period was 665 grm. This yielded 20·2195 grm. of unsaponifiable matter, from which 14·7324 grm. of coprosterol were obtained.

Total cholesterol ingested with food	14·920	Total output of coprosterol	14·7324
Ditto intake per diem	0·393	Output per diem	0·3877

Excess of intake over output per diem, 0·0053.

The loss in weight during this experiment amounts to about 82 grm. per diem. This is considerably less than the loss in Series III and IV, which is about 114 grm. and 250 grm. per diem respectively; there must, therefore, have been an absorption of cholesterol going on in the intestines during the period.

Conclusion.

It would appear from these experiments that in man, as in the case of other animals, the excretion of cholesterol in the fæces can be accounted for by that taken in with the food, provided that the body weight remains constant. If, however, a *rapid* loss in weight takes place, as in illness, the output of cholesterol exceeds the intake. Further work will, however, be necessary before this view can be regarded as fully established.

Note on the Sterol Contents of Rabbit Fæces.—In Part VIII of this series of papers we described an experiment in which we succeeded in isolating cholesterol by the digitonin method from the fæces of a rabbit which had been fed on extracted bran, but into the peritoneal cavity of which olive oil had been injected. The animal in question had lost nearly a third of its weight during the experiment. In consequence of this result it was thought desirable to submit the fæces of rabbits fed on extracted bran, but which were not losing weight, to a more careful examination. For this purpose four rabbits were fed for about ten days on bran which had previously been roughly extracted with ether. The animals remained during the experiment perfectly constant in weight. About 1200 grm. of air-dried fæces were obtained. These fæces were extracted with ether and treated in the manner already described, and yielded about 3 grm. of unsaponifiable matter in the form of a stiff oil. This oil was dissolved in alcohol and mixed with excess of digitonin in alcoholic solution. The precipitate was filtered and washed with ether to get rid of oily matter, and repeatedly boiled out with methyl alcohol, in which it proved very insoluble. The oily matter separated from the compound did not give any sterol colour reaction in chloroform solution with acetic anhydride and sulphuric acid. The digitonin precipitate, which the above treatment had not freed from traces of some fluorescent colouring matter, was finely powdered and decomposed by heating in xylene vapour. The clear xylene solution on evaporation gave a yellow, crystalline, oily solid. This was purified by repeated crystallisation from dilute alcohol, from which it separated when pure in microscopic hexagonal plates. It melted at 136°–137° C. and gave the usual sterol colour tests. The acetate, made in the usual manner with sodium acetate and acetic anhydride, crystallised from alcohol in glistening leaves. It was less soluble in alcohol than the original substance. It melted at 135°–136° C., but if heated very slowly, at about 130° C., the benzoate, made by the action of benzoyl chloride in pyridin solution, crystallised readily from strong alcohol, in which it was sparingly soluble. It melted at 142° C. to a clear liquid, which on cooling gave at the moment of solidification a brilliant green play of colours, gradually changing to brown. This substance was one of the phytosterols of the bran, which had not been eliminated in the rough extraction with ether. The same substance was obtained by Dorée and Gardner from horse dung.

No trace of cholesterol was discovered. This bears out our previous conclusion that cholesterol is not found in the normal fæces of herbivorous animals.

We are indebted to the Government Grant Committee of the Royal Society for help in carrying out this work.

Some Experiments with Arsenphenylglycin and Trypanosoma gambiense *in* Glossina palpalis.

By H. L. Duke.

(Communicated by Sir John Rose Bradford, K.C.M.G., Sec. R.S. Received September 28,—Read November 14, 1912.)

The experiments detailed below were devised with a view to investigating the action of arsenic in the form of arsenphenylglycin upon *Trypanosoma gambiense* as carried by *Glossina palpalis.* It was found convenient to deal with the subject under four separate enquiries—

I. Does the presence of arsenic in the blood ingested by a positive fly destroy the trypanosomes in that fly?

II. Does preliminary feeding of flies on blood containing arsenic have any effect on the subsequent development of trypanosomes in their interior?

III. If flies are fed on blood containing arsenic shortly after the infecting feeds on a *gambiense* monkey, are the flagellates still capable of development in the fly? If they can still develop, is the resultant strain arsenic-resistant in the blood?

IV. Has arsenphenylglycin any prophylactic action against the bite of a fly infected with *T. gambiense,* and, if so, what is the extent of this protection?

In all these experiments it was deemed advisable to feed each box of flies for two consecutive days on the monkey in order to make certain, if possible, that each fly fed. For the same reason special attention was given to each box of flies handled, with the result that it was found that flies reluctant to feed in the morning would often, if given another opportunity some hours later, bite with greater readiness. In spite, however, of all precautions, it is impossible to be absolutely certain on this point. In the great majority of cases the flies fed readily every day.

I. *Does the Presence of Arsenic in the Blood imbibed clean an Infected Fly of its Flagellates?*

To answer this query boxes of laboratory-bred *G. palpalis,* known to be infective with *T. gambiense,* were placed upon monkeys which had previously received a subcutaneous dose of arsenphenylglycin, 0·1 grm. per kilogramme body weight. The experiments were commenced 24 to 48 hours after the administration of the drug, and subsequently the period of time was increased. No change in the flagellates was discernible if the interval between the giving of the arsenic and the feeding exceeded 72 hours. The flies were dissected by Miss Robertson at varying periods after the feeds upon the arsenic monkey. I have here to express my indebtedness to her for the description of the flagellates found. It was constantly noticed in the case of flies which had fed upon a recently inoculated monkey that the walls of the intestine were unduly brittle and friable, and clean dissection very difficult. This was to a great extent avoided by starving such flies for several days after the ingestion of the arsenic blood before dissecting them. In the following experiments various changes were noted in the flagellates in the fly, due presumably to the action of the drug in the blood imbibed:—

Expt. 29.—Positive Box of *G. palpalis*.

Date.	Procedure.	Remarks.
Oct. 1—2 ……	Fed on Monkey 418 24—48 hours after administration of 0·1 grm. arsenphenylglycin per kilogramme.	
„ 3…………	Starved…………	1 fly dies; nil.
„ 4…………	Fed on clean Monkey 502…………	Monkey 502 becomes infected. 2 flies die; nil.
„ 5…………	Fed on clean Monkey 427…………	Monkey 427 becomes infected. 1 fly dies; nil.
„ 6…………	Starved and dissected…………	1 + fly found.

Description of the positive fly:—

Gut: Shows flagellates very much modified, with swollen posterior end; many dead.

Proventriculus and thoracic gut: No flagellates seen.

Salivary glands: Normal, though somewhat slight infection.

It will be noted that for two consecutive days after feeding on the arsenic monkey this fly was infective to monkeys. The condition in this fly affords strong confirmation of the theory that the salivary gland flagellates are the true infecting form.

Expt. 74.—Positive Box of *G. palpalis*.

Date.	Procedure.	Remarks.
Oct. 24—25 ……	Fed upon Monkey 488 24—48 hours after the administration of arsenphenylglycin, 0·1 grm. per kilogramme.	
„ 26 …………	Starved…………	9 flies die, among which is + Fly No. 1.
„ 27 …………	Fed on cock.	
„ 28 …………	Fed on cock…………	2 flies die, one negative, the other + Fly No. 2.
„ 29—Nov. 1…	Fed on cock.	
Nov. 2—4 ………	Fed on clean Monkey 503…………	Monkey 503 became infected. 1 fly dies; nil.
„ 5—6 ………	Starved and dissected…………	+ Fly No. 3 found.

Description of the three positive flies:—

Fly 1. *Gut:* Few altered flagellates, all dead, with swollen posterior ends, and generally altered.
Proventriculus: Nil.
Salivary glands: Only one gland obtained; this contained living and apparently quite normal flagellates.

Fly 2. *Gut and Proventriculus:* Show no flagellates, living or dead.
Salivary glands: Contained flagellates + + +, actively motile.

Fly 3. *Gut:* + + +, apparently normal.
Proventriculus: Nil.
Salivary glands: + + +, normal.

Whether the condition of this fly is due to the original gut flagellates having escaped destruction by the arsenic, or whether in the 10 days which elapsed between the arsenic feeds and dissection the gut became reinfected from the salivary glands, cannot be decided. In relation to the first alternative it is improbable that a fly would refuse to feed upon two successive days unless at the point of death.

The mortality after the arsenic feeds should be noted in this and other similar experiments.

Expt. 357.—Positive Box of *G. palpalis*.

Date.	Procedure.	Remarks.
Oct. 10—11 ...	Fed on Monkey 452 24—48 hours after administration of arsenphenylglycin 0·1 grm. per kilogramme.	
„ 12	Starved............	7 flies die; nil.
„ 13	Dissected	1 + fly found.

(This box was killed by wood-smoke instead of the usual chloroform.)

Description of the positive fly:—

Gut: Flagellates all dead, shape considerably altered, the posterior end being frequently swollen up to a marked degree.

Salivary gland: Numerous flagellates, but all dead; shape perfectly normal.

Apparently this curious condition of the gland flagellates was due to the smoke. The effect of this treatment is very marked; the glands show a dark granular appearance very different from the clear transparent state to be observed in normal specimens. This darkening of the glands proved by subsequent experiments to be a characteristic of smoke-killed flies. This condition has never been observed in flies killed with chloroform.

Note the mortality after the arsenic feeds.

Expt. 383.—Positive Box of *G. palpalis.*

Date.	Procedure.	Remarks.
Oct. 24—25 ……	Fed on Monkey 487 24—48 hours after the administration of 0·1 grm. arsenic per kilogramme.	
„ 26 …………	Starved.	
„ 27—28 ……	Fed on Monkey 493 24—48 hours after arsenic 0·1 grm. per kilo.	
„ 29 …………	Starved.	
„ 30 …………	Fed on cock………………………………	26 flies die; nil.
„ 31—Nov. 2…	Fed on clean Monkey 502 (*a*) …………	Monkey became infected. 1 fly dies; nil.
Nov. 3……………	Starved…………………………………	1 fly dies; nil.
„ 4……………	Starved and dissected……………………	+ Flies Nos. 1 and 2 found.

Description of the two positive flies:—

Fly 1. *Gut and proventriculus:* Devoid of flagellates, alive or dead.
Salivary gland: + + +, with normal active flagellates.

Fly 2. *Gut:* + +, no alteration in form observable.
Foregut: +, no alteration in form observable.
Proventriculus: Nil.
Salivary gland: + + +, normal.

In this experiment the flies were afforded an opportunity of a second feed of arsenic blood. In spite of this the salivary flagellates—the infecting form—were, it appears, unaffected. Apparently fly No. 2 illustrates re-infection of the gut from the salivary gland, as it is very improbable that any of the original gut flagellates would survive the double feeding on arsenic blood.

Note the mortality after the arsenic feeds.

Expt. 358.—Positive Box of *G. palpalis.*

Date.	Procedure.	Remarks.
Oct. 10—11 …	Fed on Monkey 438 24—48 hours after administration of arsenphenylglycin, 0·1 grm. per kilogramme.	
„ 12 ………	Starved.	
„ 13 ………	Dissected ………………………………	+ Flies Nos. 1, 2, 3, 4 found.

Description of the four positive flies:—

Fly 1. *Gut* (containing no blood): + + + active, normal.
Proventriculus: +, normal.
Salivary glands: + + +, active and normal.

This fly showed no sign of blood in its gut, and therefore presumably did not feed on Monkey 438.

Fly 2. *Gut* (empty forward with a little pale fluid posteriorly): Some tendency towards posterior swelling, but still active.
Salivary glands: + + +, active and normal.

Fly 3. *Gut* (containing altered blood): + + +, active, but some show posterior swelling.
Salivary gland: + +, apparently normal.

Fly 4. *Gut* (containing altered blood): + + +, few dead, many altered in shape but still slowly motile, others active.

The flagellates of these flies show less effect from the arsenic than is usual at this period.

It may be remarked, as justifying the conclusion that the above departures from the normal in the flagellates are due to the action of the drug, that such alteration in form and motility have never before been seen in positive flies.

The dead flies are removed every morning, and in the normal course of events the flagellates are always seen to be actively motile with no such morphological changes as those described above. Moreover, in cases in which the normal flies have been left until the flagellates are moribund, death occurs without the characteristic swelling of the posterior part always to be observed in the presence of arsenic.

Expt. 550.—Positive Box of *G. palpalis*.

Date.	Procedure.	Remarks.
Jan. 31—Feb. 1 ...	Fed on Monkey 632 48—72 hours after administration of arsenphenylglycin, 0·1 grm. per kilogramme.	
Feb. 2	Starved.	
„ 3	Dissected	+ Flies 1 and 2 found.

Description of the two positive flies:—

Fly 1. *Gut:* + + +, normal (no traces of blood seen).
Salivary glands: + + +, normal.

Fly 2. *Gut:* + +, a few are altered as described above.
Salivary gland: + + +, normal.

A very slight effect appears to have been exerted in Fly No. 2 by the arsenic. Fly No. 1 either fed very slightly or not at all on Monkey 632.

Expt. 567.—Positive Box of *G. palpalis.*

Date.	Procedure.	Remarks.
Jan. 20—21 ……	Fed on Monkey 620 48—72 hours after administration of arsenphenylglycin, 0·1 grm. per kilogramme.	
„ 22—26 ……	Starved.	
„ 27 …………	Dissected ………………………………	1 + fly found.

Description of the positive fly:—

Hindgut: Nil.

Posterior part of thoracic gut: Normal, + + +.

Anterior part of thoracic gut: Nil.

Proventriculus: Nil.

Salivary gland: + + +, normal.

From the above experiments it will be seen that the flagellates in the gut of flies fed upon a monkey within 24 to 48 hours of the administration of arsenphenylglycin in doses of 0·1 grm. per kilogramme are markedly affected. The flagellates in the salivary glands are apparently not injured in any way, nor does the fly lose its power of infecting. This evidence supports the theory that the salivary gland flagellates are the normal infecting agents. In a paper shortly to be published, Miss Robertson brings forward a further mass of evidence to support this conclusion. There is no reason to doubt that the secretion of the salivary gland is poured out into the wound made by the fly's proboscis at the commencement of the act of feeding. Whether or not this process is repeated during the course of feeding cannot well be determined. These functions of the salivary gland and its contained flagellates are well borne out by the interrupted feeding experiment to be referred to shortly.

It is plain that any attempt to clean a positive fly of its flagellates by feeding it upon an animal whose blood contains arsenic will fail, as the gland flagellates will not come into contact with the drug.

II. *Has the Preliminary Feeding of Flies on Arsenic-containing Blood any Effect on the Subsequent Development of the Flagellates in their Interior?*

The first pair of experiments, Nos. 336 and 337, devised to elucidate this point proved fruitless, as no positive flies were found either in the arsenic box or its control.

Expt. 712.

Date.	Day of expt.	Procedure.	Remarks.
Apr. 15	—	Fed on Monkey 708 48 hours after the administration of arsenic, 0·1 grm. per kilogramme.	
„ 16—17	1—2	Fed on Monkey 711 24—48 hours after the administration of arsenphenylglycin 0·1 grm. per kilogramme.	
„ 18	3	Starved.	
„ 19—20	4—5	Fed on Monkey 597, which shows *T. gambiense* +.	
„ 21	6	Starved.	
„ 22—May 20	7—35	Fed on cock.	
May 21—22	36—37	Starved and dissected	3 + flies found out of 112 dissected = 2·6 per cent.

Expt. 713 (control).

Date.	Day of expt.	Procedure.	Remarks.
Apr. 15—17	1—2	Fed on normal monkey.	
„ 18	3	Starved.	
„ 19—20	4—5	Fed on Monkey 597 (*T. gambiense* +).	
„ 21	6	Starved.	
„ 22—May 20	7—35	Fed on cock.	
May 21—22	36—37	Starved and dissected	8 + flies found out of 69 dissected = 11·5 per cent.

From these two experiments, as far as any conclusion can be drawn from such limited evidence, it would appear that the initial arsenic feeds influence unfavourably the subsequent development of the flagellates in the fly.

III. *Does Feeding on Arsenic-containing Blood, immediately after the Infecting Feeds, prevent the Subsequent Development of Flagellates in the Fly, or does it result in the Production of an Arsenic-fast Strain?*

Expt. 416.

Date.	Day of expt.	Procedure.	Result.
Sept. 30—Oct. 1	1	Fed on Monkey 199 (*T. gambiense* +).	
Oct. 2	2	Fed on cock.	
„ 3	3	Starved.	
„ 4—Nov. 20...	4—51	Fed on Monkey 426...	Monkey 426 received 0·1 grm. per kilogramme arsenphenylglycin on Oct. 3. It eventually became infected.
Nov. 21—22	52—53	Starved and dissected	1 + fly out of 82 dissected = 1·2 per cent.

Monkey 426 became infected on November 27. On December 4, when trypanosomes were showing + + + +, it received 0·1 grm. arsenphenylglycin per kilogramme. Trypanosomes disappeared the following day and were not seen again, although the monkey was examined daily until death on January 9.

Expt. 417.

Date.	Day of expt.	Procedure.	Remarks.
Sept. 30—Oct. 1...	1	Fed on Monkey 199 (*T. gambiense* +).	
Oct. 2	2	Fed on cock.	
" 3	3	Starved.	
" 4—25	4—25	Fed on cock.	
" 26—Nov. 9	26—40	Fed on Monkey 494	Monkey 494 becomes infected.
Nov. 10—21	41—52	Fed on cock.	
" 22—24	53—55	Starved and dissected	3 + flies out of 103 dissected = 2·8 per cent.

It is possible that the + fly in Experiment 416 did not feed upon Monkey 426 on the first day after the arsenic feeding. The strength of the drug to which the newly-imbibed trypanosomes were subjected would thus be reduced. In any case, the trypanosomes in Monkey 426 at the end of the experiment showed no resistance to arsenic.

Experiments 428 and 429, in each of which 89 flies were employed, and Experiments 350 and 351, in which 121 and 77 flies were used respectively, were useless, as no flagellates were found in either the arsenic boxes or their control.

Expt. 714.

Date.	Day of expt.	Procedure.	Remarks.
Apr. 17—18	1	Fed on Monkey 597 (*T. gambiense* +).	
" 19	2	Starved.	
" 20—May 20	3—33	Fed on cock.	
May 21	34	Dissected	7 + flies found out of 61 dissected = 11·4 per cent.

Expt. 715 (control).

Date.	Day of expt.	Procedure.	Remarks.
Apr. 17—18	1	Fed on Monkey 597 (*T. gambiense* +).	
„ 19	2	Starved.	
„ 20—25	3—8	Fed on cock.	
„ 26—27	9—10	Fed on Monkey 724	Monkey 724 received 0·05 grm. per kilogramme arsenphenylglycin on April 25.
„ 28	11	Starved.	
„ 29	12	Fed on Monkey 724.	
„ 30—May 20	13—33	Fed on cock.	
May 21	34	Dissected	1 + fly found out of 58 dissected = 1·8 per cent.

This positive fly showed only a slight gut infection; no flagellates were present in salivary glands or proventriculus. This is an extremely backward state of development for the age of the fly.

From these experiments it appears that ingestion of arsenic blood immediately after the infecting feeds checks subsequent development of flagellates in the fly.

IV. *To Investigate the Prophylactic Properties of Arsenphenylglycin against the Bite of* G. palpalis *Infected with* T. gambiense.

In a paper summarised in the 'Sleeping Sickness Bulletin,' No. 24, vol. 13, Mesnil and Kérandel give some experiments dealing with the prophylactic action of arsenphenylglycin against inoculation of *T. gambiense* into monkeys. The following experiments were undertaken on similar lines, but only the smaller doses used by the French observers were employed. Thus in the first series 0·1 grm. of the drug per kilogramme was inoculated subcutaneously; in the second 0·05 grm. per kilogramme. Such proportions when applied to man involve a relatively enormous dose of the arsenphenylglycin. Nevertheless, although the practical application of the results given below may be very limited, the much greater potency of the drug against the fly infection is of considerable interest.

In undertaking such experiments with living flies an obvious difficulty arises in the uncertainty as to whether the infected fly has fed upon the experimental animal. To insure this as far as possible without at the same time vitiating the exactness of the experiment, each box of flies was placed upon the monkey for two consecutive days, and on each day repeated efforts were made if the flies showed reluctance to feed. In the great majority of cases a day's starvation before the experiment commenced insured vigorous feeding of all the flies. Again, considerable time must often elapse before an infected box of flies is obtained, a large number of boxes having to be rejected after some 30 days' observation, owing to the flies proving non-infective. It was thus found necessary on several occasions to employ a positive box for several different experiments. Also, owing to the great sacrifice of experimental animals involved in testing the infectivity of each box, it was sometimes found necessary to employ untested boxes, relying on the ultimate dissection of the flies to prove their infectivity. Fortunately two monkeys were available which proved highly infective to flies. Thanks to a large number of experiments carried out by Miss Robertson, in which these two animals were employed for the infecting feeds, it became evident that flies infected from either of these monkeys were invariably infective by the 30th day of the experiment, and often several days earlier. This fact, together with the observation derived from Miss Robertson's experiments—that the infectivity of the fly coincided with the invasion of the salivary glands by the flagellates—made it possible to employ untested boxes as above described. All such boxes were kept until the 32nd day, and then assumed to be infective. If on subsequent dissection no + flies were found, then the box was ignored. In the following tables it is stated whether or not a tested box was employed. It will be noted that in positive Experiment 757 only untested boxes were used.

The following facts must also be considered as having an important bearing on the present series of experiments :—

1. The mere introduction of the proboscis of a positive fly into the skin of its victim can produce infection. This was proved by Fraser and myself by an experiment in which interrupted feeding was employed, and is in agreement with the contention that the salivary gland flagellates are responsible for infection. Thus a fly need not have actually extracted any blood to have infected the monkey.

2. A single positive fly can infect a clean monkey on three consecutive days. This experiment was reported by me to the Royal Society in April, 1912. This applies to the occasional employment of the same box in different experiments on consecutive days.

3. The facts recorded in Section I relating to the persistence of the salivary gland flagellates and the infecting power of a fly, even after the gut has been cleared by arsenic feeding.

The actual experiments may now be considered. The headings of the columns are self-explanatory. The variation in the number of positive flies on the two days of an experiment is due to the necessity of apportioning

Expt. No.	Period elapsing between the administration of the arsenic and the application of the positive flies.	Number of experiments fed on the monkey.		Number of positive flies to which the monkey was exposed.		Duration of the examination of the monkey.	Result.	Remarks.
		First proved infective on a clean monkey.	Not so tested.	First day.	Second day.			
	hours.					days.		
418	24—48	1		1	1	52	—	
438	24—48	1		4	4	Died after 9 days	?	Received second dose of approximately 0·07 grm. per kilogramme three days after first dose. Died of arsenic poisoning.
452	24—48	1		1	1	45	—	
487	24—48	1		2	2	40	—	
493	24—48	1		2	2	41	—	
524	48—72	1		2	2	11	?	Old monkey; showed severe local reaction; killed.
620	48—72	1		1	1	50	—	
632	48—72	1		2	2	60	—	
654	72—96	1		1	1	60	—	
694	72—96	2		3	3	62	—	
661	96—120	1		6	6	50	—	
662	96—120	1		2	2	42	—	These two flies died on 22nd, and, from the condition of their gut, apparently had not fed on 662. They were placed on monkey on 21st and 22nd.
692	96—120	1		2	2	62	—	
695	96—120	1		2	2	63	—	
689	120—144	2		5	5	67	—	
708	144—168	3		12	12	61	—	
711	168—192	2	1	5	5	54	—	
718	192—216	6	1	12	6	60	—	
694 (*a*)	216—240	2		3	3	55	—	Blood injected on the 55th day into white rat, with negative result.
782	264—288		3	3	3	49	—	Blood injected into white rat on 30th day of experiment, with negative result.
757	312—336		2	4	4		+	Incubation period 10 or 11 days.

the available positive boxes, so that each monkey had every chance of infection. In many cases boxes had to be ignored owing to their proving negative on dissection. Each monkey was kept under observation for a considerable time in consideration of the enormous incubation period reported in certain experiments by Mesnil and Kérandel where trypanosomes appeared only after 39 days. No such phenomenon occurred here.

Thus a dose of arsenphenylglycin of 0·1 grm. per kilogramme will protect a monkey against infection by positive *G. palpalis* if given within 12 days before exposure.

Expt. No.	Period elapsing between the administration of the arsenic and the application of the positive flies.	Number of experiments fed on monkey.		Number of positive flies to which monkey was exposed.		Duration of examination of monkey.	Result.	Remarks.
		First tested on a clean monkey and proved infective.	Not so tested.	1st day.	2nd day.			
	hours.							
720	72— 96	2		4	4	56	–	
719	96—120		3	10	10	61	–	
724	120—144	2	1	5	5	50	–	
774	144—168		4	4	4	65	–	
775	144—168		4	4	3	65	–	
749	168—192	2	2	8	5		+	Incubation period 10 or 11 days.
748	192—216	1	2	4	1		+	" "

Thus a dose of arsenphenylglycin of 0·05 grm. per kilogramme will protect a monkey from infection by positive *G. palpalis* if given within seven days of exposure. Using the inoculation method of infection, Mesnil and Kérandel proved that 0·05 grm. of this drug per kilogramme was capable of protecting a monkey from infection up to within three days of the administration of the drug.

Conclusions.

I. By feeding a *G. palpalis* which is infective with *T. gambiense* on an animal whose blood contains arsenic administered within 24 to 48 hours, the gut flagellates in the fly may be destroyed. Those of the salivary glands, however, which are the infecting forms, are unaffected. It is highly probable that the gut may become repopulated with flagellates from the salivary glands.

II. Preliminary feeding of flies on arsenic-containing blood has a deterrent effect on the subsequent development of *T. gambiense* ingested within 24 to 48 hours after the arsenic blood.

III. The feeding of flies on arsenic-containing blood immediately after the imbibition of *T. gambiense* usually prevents further development of the trypanosomes in the fly. In the event of development occurring the strain produced is not arsenic-fast.

IV. Arsenphenylglycin exerts a prophylactic effect in a monkey against infection with *T. gambiense* by positive *G. palpalis*; this effect varies with the dosage employed, and is considerably greater than when the trypanosomes are introduced by direct inoculation of infected blood.

On a Gregarine—Steinina rotundata, *nov. sp.*—*Present in the Mid-Gut of Bird-Fleas of the Genus* Ceratophyllus.

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[Plate 1.]

The Gregarine described in the following account was first observed by one of us, in autumn, 1909, in the alimentary canal of fleas—*Ceratophyllus styx*, Rothschild—from a sand-martin's nest.* We have since collected, from several localities in the Scottish Lowlands, numerous larval, pupal, and adult specimens of *C. styx*, in which we have observed the various phases of development of the parasite. We have also dissected about 500 fleas of other species, in order to determine whether they also harboured the Gregarine which we had found in *C. styx*. The species examined were *C. farreni*, Rothsch., from nests of the house-martin; *C. gallinæ*, Schrank, from nests of the blue-tit,† the blackbird, the thrush, and the robin;

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* The Rev. J. Waterston had previously reported that he had noticed in a specimen of *C. styx*, mounted whole, after having been partially cleared in caustic potash, a rounded body of doubtful nature. He kindly obtained for us other examples of *C. styx* from the locality in which he had collected the specimen referred to. These proved to contain the vegetative phases of the parasite described in this account.

† Most of the batches of *C. gallinæ* examined were from the nests of the blackbird and the thrush, and were found to be either free from Gregarines or infected in very small degree only. But a heavily infected colony was found in the nest of a blue-tit, taken

C. gallinulæ, Dale, from nests of the chaffinch and blackbird; *Ctenocephalus canis*, Curtis, from rabbits; and *Pulex irritans*, Linn., from a dog-kennel. All the series of *C. styx* dissected proved to be heavily infected, the percentage carrying Gregarines ranging from 65 to 100. The degree of infection was much less in the case of *C. farreni* and *C. gallinæ*—about 7 per cent. in the former and 5 per cent. in the latter—while the remaining species above mentioned were found to be free from Gregarines.

There are four previous records of the occurrence of Gregarines in fleas. R. Leuckart* mentioned that he had observed Gregarines in the gut of flea-larvæ, but gave no other information regarding them. E. H. Ross† found specimens, for which he proposed the name *Gregarina ctenocephali canis*, in the alimentary canal of adult examples of the dog-flea—"*Ctenocephalus serraticeps*," collected in Egypt. He described the trophozoites, which were frequently found in pairs, as rostrated and pear-shaped, and gave a brief account of the life-history of the parasite, at least, of those phases which occur in the adult flea. The description given is, however, not sufficient to permit the organism to be identified with certainty, but several of the features mentioned by Ross indicate that the "*Gregarina*" which he observed is distinct from the organism which forms the subject of the present communication. The third record is by Wellmer,‡ in the following terms: "*Actinocephalus parvus*, n. sp., im Darm der Larven von *Ceratophyllus fringillæ* (Wlk.) und *C. gallinæ* (Schrank). Das auf einem kurzen Halse des Protomerites sitzende, lange beständige, scheibenförmige Epimerit, trägt 8 Haken; Maximallänge der Sporonten 140 μ." This Gregarine is markedly different from ours in the nature of the armature of the epimerite and in other respects. The fourth record, by C. Strickland,§ relates to a Gregarine which lives for part of its life in the alimentary tract of the larva of *Ceratophyllus fasciatus*, and "for the other part lives freely in the excrement of this host." Strickland states that "the form of its epimerite and spores‖ precludes us from placing it in any of the known families of

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from a hole in a wall. This nest, in regard to protection and supply of moisture, was comparable to those of the sand-martin, and, like the latter, was evidently favourable to the survival of the spores of the Gregarine and to the chances of infection of the flea-larvæ.

[*] 'Arch. f. Naturgesch.,' 26 Jahrg., 1861, Bd. 2, p. 263.

[†] 'Ann. Trop. Med.,' Liverpool, 1909, vol. 2, pp. 359–363.

[‡] 'Zool. Anz.,' 1910, Bd. 35, p. 533. Wellmer has since figured a cephalont and a sporont of *Actinocephalus parvus* in his Inaug. Diss., 'Sporozoen Ostpreussischer Arthropoden,' Königsberg, 1911, p. 33. This memoir is also published in 'Schr. Physik.-ökonom. Ges.,' Königsberg, Jahrg. 52, 1911, Heft 2.

[§] 'Camb. Phil. Soc. Proc.,' 1912, vol. 16, pp. 460, 461.

[‖] No details of the forms of these are given.

cephaline Eugregarines, which have spores unarmed with spines." He proposes to name the Gregarine *Agrippina bona*, and to refer it to a new family—the Agrippinidæ. As this Gregarine exhibits features so striking as to require for its reception a new family, it is evidently different from that described below, for the latter is referable to a well-known genus, *Steinina*. *Agrippina bona* differs from the new species of *Steinina* described in the following pages, not only in certain points of structure, but in being restricted to the larval gut and fæces, for, in *S. rotundata*, the trophozoites, although occurring in the larva, reach their full size only in the adult flea.

Description of Steinina rotundata, *and of its Life-History.*

The life-cycle of this Gregarine has been traced almost exclusively in specimens of *Ceratophyllus styx*. The early trophic phases of the Gregarine occur in the larvæ of this flea, and are either attached to the wall or lie free in the lumen of the mid-gut. We have carefully examined the freshly dissected mid-gut, and also serial sections of the gut of many infected larvæ and adults, but we have not been able to identify an intra-cellular phase of the Gregarine. This stage, if it exists, will be extremely difficult to recognise with certainty, owing to the close resemblance between the young Gregarines and the smaller cells about the bases of the gastral epithelium, especially in the neighbourhood of the numerous crypts of regeneration. If there be an intra-cellular stage, it is of brief duration only, for we have observed a number of very young, ovoid trophozoites, 10 μ long and 5 μ broad,* attached by their narrower ends to the epithelial cells and hanging into the lumen of the mid-gut (Plate 1, fig. 1).

Average specimens of the Gregarine, from the mid-gut of young adult fleas, are 45 to 70 μ long and 30 to 50 μ broad, but, when full grown, the parasite attains a length of 180 μ and a breadth of 70 to 80 μ. It is usually differentiated into three regions—epimerite, protomerite, and deutomerite.

The epimerite varies considerably in shape. It often has the form of a blunt and flattened cone (figs. 2A, 3, 5, 6), on the apex of which a small pointed process may be present (fig. 3). In some specimens the epimerite is discoidal or saucer-shaped (fig. 4), its edge being slightly upturned (that is, away from the protomerite). The margin of the epimerite is rarely entire; sometimes it is lobate, but more usually it is fringed with short processes, rounded or pointed at their tips. These processes are, in some specimens, placed at almost regular intervals and form a single series, while in others they are less evenly arranged, and may be in two or three irregular rows (figs. 2, 4, 5, 6).

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* The sporozoite is about 10 μ long and 1·5–2 μ broad (see p. 36).

The protomerite is narrowed in front into a neck-like region, which bears the epimerite, but the "neck" is usually short, and it is very short in the case of specimens lying free in the gut. The protomerite and deutomerite together form a mass which, in the smaller trophozoites, is usually oval, or, in some cases, nearly spherical, but in the largest examples is almost pear-shaped (fig. 8).

These two regions are separated internally by a thin septum, but the external surface does not bear any well-marked groove indicating the line of separation. In the few cases in which an external groove was present, it was faint. The septum is seldom equatorial in position; it is generally nearer the epimerite.* Below the thin epicyte (cuticle) there is a peripheral layer of ectoplasm 2 to 3 μ in breadth, which is clear, as it contains few granules. This layer is thicker at the distal end of the deutomerite. The protoplasm of the "neck" is generally homogeneous, like the ectoplasm. In this region faint longitudinal striæ, probably myocyte fibrillæ (myonemes), are present (fig. 9). Such striæ were not observed in other portions of the Gregarine. The endoplasm is very fluid, and contains numerous grey, refringent, nutrient granules, the largest of which are about 2 μ in diameter. Granules are much more abundant and coarser in the endoplasm of the deutomerite than in that of the protomerite. The nucleus is invariably situated in the deutomerite. In the fresh condition it is a clear vesicle with regular outlines, and contains from one to four karyosomes. The latter are usually homogeneous, but sometimes they are vacuolated (figs. 4, 5, 9), and in several cases they were observed to contain spherules of more refringent nature (fig. 6), composed of denser chromatin.

The Gregarines generally occur in considerable numbers; in one case 75 were present in the mid-gut, but they are not found in pairs, except at the moment of encystment.

When full grown the Gregarines become associated in pairs, preparatory to the formation of gametes. We have made numerous attempts to obtain the early stages of this association, but in the youngest met with the organisms had formed the common cyst wall, and the peripheral protoplasm of each individual was already raised into the rounded masses, which would soon have become gametes. We conclude that the association of the two individuals, and the formation of the cyst wall and of the gametes succeed each other very rapidly in this species.

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* We have seen a considerable number of trophozoites of various sizes up to 40 μ in length, in which the epimerite was wanting. These were lying free in the cavity of the mid-gut. They were ovoid in form (fig. 2, B), and the septum was almost invariably absent. Whether such specimens can subsequently develop a new epimerite, and re-attach themselves to the gut-wall, was not ascertained with certainty, but it appears probable that the smaller ones, at any rate, may do so.

A period of about two months elapses between the time of infection and the first appearance of cysts. This was ascertained by examination at regular intervals of the series of larval, pupal, and adult fleas, which were infected in the laboratory (see below, p. 36). Young larvæ, recently hatched, were collected on August 5, 1910, and placed, on August 8, in the *débris* of a nest known to be infected with the Gregarine. During the fortnight following August 10, 14 larvæ were examined; in all of them early trophic phases of the parasite were present. On August 25, cocoons were noticed; they contained either pupæ or scarcely formed adults. On August 26, 27, and 30, young adult fleas were removed from the cocoons and dissected, but only early vegetative phases of the Gregarine were found. Other adult fleas from this series, dissected at intervals during September, also exhibited trophic phases of the parasite. The first cysts were observed on October 15, that is, more than nine weeks after the larvæ had been placed in the infective environment.

The cysts are approximately spherical (fig. 10), and range from 110 to 185 μ in diameter.* When first formed they are translucent and grey, but they become finally yellow or yellowish-brown in colour. A mucous portion of the cyst envelope (*i.e.* the epicyst) appears to be absent, but the endocyst is well developed. When freshly formed, and for a considerable time afterwards, the endocyst is about 8 μ thick, but in very old cysts it has evidently undergone condensation, becoming thinner (1 to 2 μ), yellow, and less translucent. The cyst envelope is plain, that is, not sculptured in any way. The two individuals associated in each cyst give rise to gametes of apparently similar form, but we have not investigated the gametes, nor have we observed their fusion.

Each cyst soon contains numerous spores, among which are one or more masses of residual protoplasm, situated in the centre of the cyst. Each spore is oval, and is 11 to 12 μ long and about 7 μ broad (fig. 11). The epispore is closely applied to the endospore and exhibits two polar thickenings. Each spore, when ripe, contains eight sporozoites and a small amount of axial residual protoplasm.

Cysts—1 to 14 in number, in different individuals—occur lying freely in the mid-gut of the flea. They cannot escape entire from the digestive tract, as the lumen of the intestine is much too small to permit their passage (fig. 10). When fully ripe, the cysts burst in the mid-gut, and their contained spores are discharged with the fæces of the host. We have seen, in the mid-gut of an adult flea, a ruptured, yellow cyst, from which some of the spores (containing fully formed sporozoites) had escaped. The spores are dropped by the host, and are ingested by the flea-larvæ feeding among the *débris* in the nest. We have several times found young trophozoites in the mid-gut of larvæ which had been hatched only two or three days.

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* We have occasionally seen small cysts, about 50 μ in diameter, but these did not contain spores. Probably each cyst resulted from the encystment of a single individual, and none of them would have developed further. The contents of two were examined, and were found to have degenerated.

During the course of our experiments we procured a heavily infected series of adult fleas, by rearing them in the laboratory, from larvæ which we placed in an old nest, in which a considerable number of infected fleas had been kept and had died. Fourteen of these larvæ were dissected, and all proved to be infected; the rest were allowed to grow into adults, 11 of which were dissected, and were all found to contain Gregarines. As six adult fleas from the original nest in which the larvæ were found, proved on dissection to be uninfected, we think that we may reasonably attribute the occurrence of Gregarines in every example of our laboratory-bred series to the fact that the larvæ had been reared in material which offered them a ready means of infection.

Ripe spores, when placed in the fluid obtained by puncture from the mid-gut of larval fleas, soon exhibit changes. The polar caps of each spore become swollen and detached, leaving the rest of the spore like a barrel open at both ends, from which the eight sporozoites escape.

Each sporozoite is, when extended,* about 9·5 to 10·5 μ long, and 1·5 to 2 μ in diameter at its widest part (figs. 12, 13). It has a slender, finger-shaped, and mobile "rostrum," the organ by means of which fixation to the gastral epithelium of the host is accomplished. The protoplasm of the sporozoite is finely granular, and the nucleus is visible in the living condition. On fixing and staining the sporozoites, the protoplasm was found to stain almost homogeneously, except at the base of the "rostrum," where it was, in most cases, coloured rather more deeply. The nucleus is large and vesicular. Associated with its thick membrane are a few (four to six) small and deeply stained chromatin masses. A karyosome is not present at this stage.

We have not been able to observe the attachment of the sporozoite to the epithelium of the larval gut, nor (as stated on p. 33) to determine whether or not this is followed by an intracellular stage. There is evidence, however, that the sporozoite, on fixation to the host, becomes more ovoid, that is, shorter but broader, for, on the wall of the mid-gut of a young larva (1·5 mm. long), we found a very young trophozoite, about 5 μ long and 3 μ broad. Whether this was attached to, or was within, an epithelial cell could not be ascertained. The karyosome, which was already developed in this young trophozoite, soon attains a considerable size. The septum, dividing the protomerite from the deutomerite, appears later, when the organism has reached a length of about 30–40 μ.

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* The body of the sporozoite can be bent into a curve or hook (fig. 12).

During the early phases of growth in the larval gut, the trophozoite may relax its hold of the epithelium, and may, later, renew its attachment, but whether all the detached trophozoites succeed in refixing themselves is not clear (see footnote, p. 34).

Systematic Position.

The characters of the vegetative phases, cysts, spores, and sporozoites of the Gregarine described in the preceding pages agree closely with those of the genus *Steinina*, Léger et Duboscq,* which was defined in the following terms: "Polycystidée de la famille des Actinocéphalides, caracterisée par un épimérite constitué d'abord par un court prolongement digitiforme et mobile et plus tard par un bouton aplati. Développement à phases fixées pouvant alterner avec des phases libres. Kystes sans sporoductes, à sporocystes biconiques, fortement ventrus." The only feature in which the organism described by us deviates from this diagnosis is the shape of the spore, which is not strongly inflated. However, the difference in regard to this character is so small that we do not hesitate to refer our Gregarine to the genus *Steinina*. This genus has hitherto been represented by a single species —*S. ovalis* (Stein)—recorded† from the intestine of the larva (meal-worm) of *Tenebrio molitor*. Our specimens differ from this species in (1) the shape of the spores, which are less strongly inflated; (2) the size of the spores, which are 11 to 12 μ long and about 7 μ broad (in *S. ovalis* they are 9 μ long and 7·5 μ broad); and (3) the younger trophic phases are usually less elongate and more oval or spherical, and the sporonts ("sporadins") are more pyriform. These differences seem to us to be sufficient to justify the formation of a new species, for which we propose the name *Steinina rotundata*, for the specimens described in this paper.

Steinina rotundata, n. sp.—Near *S. ovalis*, Léger et Duboscq, but the spores are larger and less strongly inflated than those of the latter. In the younger trophozoites, the protomerite and deutomerite together usually form an oval or sub-spherical mass, but later the organism becomes more or less pyriform. Adults solitary, except when association in pairs occurs preparatory to the formation of gametes. Cysts spherical, 110 to 185 μ in diameter, without sporoducts. Spores oval, 11 to 12 μ long, and about 7 μ broad.

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* 'Archiv f. Protistenkunde,' 1904, vol. 4, pp. 352—354, 2 figs.

† See Léger and Duboscq, *op. cit.*, in which the earlier records are cited. *S. ovalis* has also been recorded from the gut of the meal-worm by Kuschakewitsch ('Archiv f. Protistenkunde,' 1907, Suppl. vol. 1, p. 203) and Pfeffer (*op. cit.*, 1910, vol. 19, p. 108), and its cycle of development has been studied by Mavrodiadi ('Procès-verbaux Soc. Natural. Varsovie,' 1909, vol. 21, pp. 106—118).

Habitat.—The mid-gut of certain species of bird-fleas of the genus *Ceratophyllus.*

DESCRIPTION OF PLATE.

List of Reference Letters.

A., anus; Ep., epithelium of mid-gut; G., gizzard; Int., intestine; K., karyosome; M., muscle-fibre; M.G., mid-gut; M.T., Malpighian tube; N., nucleus; Œ., œsophagus; R., rectum; R.P., rectal papilla.

All the figures relate to *Steinina rotundata*, nov. sp., from the mid-gut of *Ceratophyllus styx*, Rothsch. Figs. 1, 9, and 13 are from stained preparations; the rest were drawn from living specimens.

Fig. 1.—Section of a portion of the epithelium of the mid-gut of a young larva, 3 mm. long, to which a young trophozoite is attached.

Fig. 2.—Two later trophic phases, from the mid-gut of a young adult flea. A exhibits the division into epimerite, protomerite, and deutomerite; B was lying free in the cavity of the gut and was not differentiated into regions.

Figs. 3, 4, 5, 6, 7.—Trophozoites from an adult flea. On dissection of the mid-gut these specimens became free from the gut-wall. Note the varying form of the epimerite, the vacuolation of the karyosome in figs. 4, 5, and the denser chromatin-spherules in the karyosome of fig. 6.

Fig. 8.—Almost full-grown adult (sporont or sporadin), found attached to the gut-epithelium of an adult flea. Note the magnification is only half that of figs. 3 to 7 and 9.

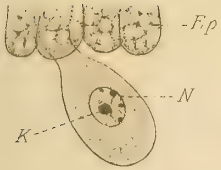
Fig. 9.—Section of a portion of the wall of the mid-gut of an adult flea, in the epithelium of which the epimerite of a well-grown trophozoite is deeply embedded.

Fig. 10.—Outline of the gut—from the œsophagus to the anus—of an adult flea and of its contained parasites. Only the proximal portions of the Malpighian tubes are shown. The five cysts contained fully formed spores. The four trophozoites were similar in form to that shown in fig. 2, B.

Fig. 11.—Outline of the wall of a spore.

Fig. 12.—A sporozoite, immediately after its liberation from the spore, drawn while living. Note the slender, mobile "rostrum." The opposite end of the sporozoite was bent into the form of a hook.

Fig. 13.—Sporozoites, from a preparation fixed with osmic vapour and stained with Leishman's stain. A, B, C, D, represent typical sporozoites; those lettered E, F, G, H are less usual forms.



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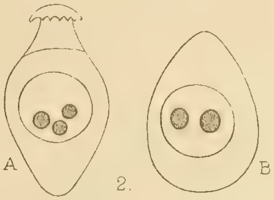

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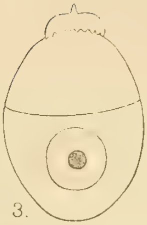

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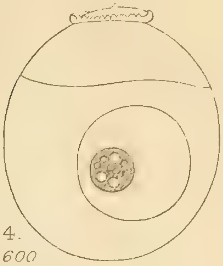

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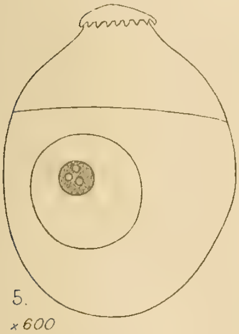

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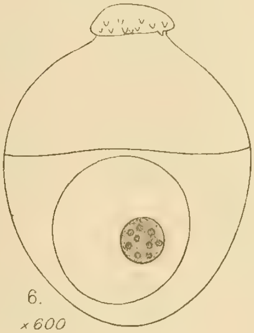

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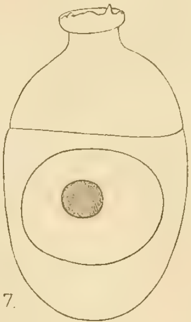

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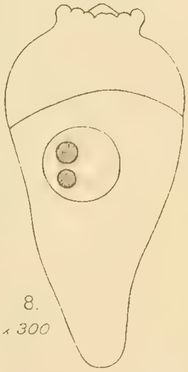

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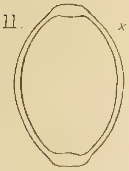

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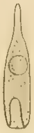

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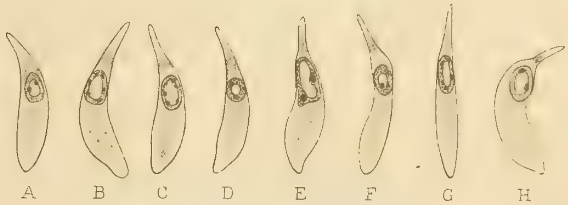

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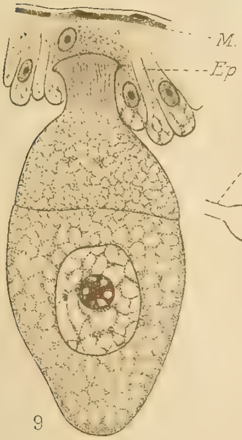

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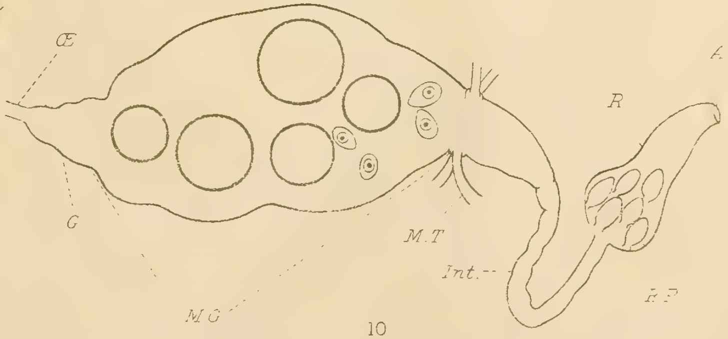
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The Size of the Aorta in Warm-Blooded Animals and its Relationship to the Body Weight and to the Surface Area Expressed in a Formula.

By Georges Dreyer, William Ray, and E. W. Ainley Walker.

(Communicated by Francis Gotch, F.R.S. Received September 30,—Read December 5, 1912.)

(From the Department of Pathology, University of Oxford.)

In recent years it has become increasingly evident that many of the most important problems of physiology and of experimental pathology cannot be investigated in a satisfactory manner until accurate data have been made available regarding the quantitative differences which are exhibited by the organs, tissues, and fluids of the body in normal animals of different species and of varying weights. Results obtained with animals of any given weight cannot be applied, even within one and the same species, to yield conclusions regarding animals of a different weight until it has been determined with precision how the various organs and tissues of the body are related to the size of the individual. Moreover, it will not be possible to compare one species with another, or to apply the results deduced from any given species to any other species of animal, until we can establish the existence of some kind of quantitative correlation between the measurements in different species. That this will prove to be possible seems likely from an examination of the results already obtained by us in studying the various factors which influence the circulatory system and determine the size of the heart (1).

In connection with our study of the blood and cardio-vascular system under normal and pathological conditions, it was shown that the blood volume of normal animals of any given species is proportional to their body surface, and follows the formula $B = W^n/k$, where k is a constant for the species and n is approximately 0·70–0·72 (2), (3). Accordingly it became of interest, in view of the theories which have been put forward regarding the volume of the blood and the size of the aorta in chlorotic conditions, to endeavour to determine how the size of the aorta is related to the weight of the individual in any given species of animal.

For this purpose we have made a series of measurements of the aorta in various mammals and birds (4). In the case of the birds some of the measurements which we have made use of were carried out by Mr. H. K. Fry in connection with other work undertaken in the department in collaboration with one of us (G. D.), but not yet published.

All the animals used were strong and healthy and in good condition. They had been kept in the laboratory under as equable conditions as possible as regards food, etc., and, of course, had not been employed in any previous experiment. Pregnant animals were naturally excluded.

Methods.—The animals were killed, the aorta divided just above the semilunar valves (care being taken that the section should be accurately transverse), and a portion removed from the body by cutting through the aortic arch after careful dissection. The proximal end of this portion of the aorta was then measured in the following manner:—The severed artery is placed in a special holder, closing with parallel limbs by means of which a uniform and gentle pressure is applied to the vessel until its lumen is just obliterated, and the walls meet in a straight line along the middle of the closed vessel. This straight line is then measured with fine-pointed compasses and the measurement pricked off on a piece of hard glazed drawing paper. This length represents half the internal circumference (πr) of the vessel. The external measurement is recorded in a similar manner. The vessel is then released completely and the process repeated again and again until from four to eight independent measurements (both internal and external) have been recorded. Great care is taken to avoid squeezing the vessel unduly. At the same time sufficient pressure must be applied to ensure that the walls lie quite flat against each other. The lengths thus pricked off upon the paper form a permanent record, and are subsequently measured by means of special callipers, fitted with a vernier scale which allows readings to be made to 1/20 mm. From the observed πr the radius and the sectional area of the vessel are determined. The internal measurements are those made use of in the present communication, which deals only with the capacity of the vessel.

It must be observed that while the measurements which we have made give a reliable value for the (internal) circumference of the aorta within each species, they do not necessarily afford an absolute measure of the size of the vessel in these animals at any given phase of the circulation during life.

The method here made use of was selected because it proved to yield the best results in our hands, particularly in the case of small animals. But it is open to considerable experimental error. This source of fallacy, however, is greatly reduced by multiplying the number of individual observations of each artery. It also diminishes greatly with increasing experience.

Own Observations.—In all the tables the body weight recorded is the natural weight (in grammes) of the animal immediately before it was killed, and consequently includes the weight of the contents of the alimentary canal, *i.e.* it is "Rohgewicht." The aortic radius is calculated in millimetres and the sectional area in square millimetres from the observed πr.

Table I.—Guinea Pigs (individuals).

No.	Sex.	Body weight.	Radius of aorta.	Area of aortic cross-section.	$k = W^{0·71}/A$.	Aortic cross-section as percentage of body weight.	Cross-section calculated. $A = W^{0·71}/k$. ($k = 24·9$.)	Difference between cross-section calculated and observed.	Cross-section calculated as percentage (0·78) of body weight.	Difference between cross-section calculated and observed.
		grm.	mm.	sq. mm.				per cent.		per cent.
1	♂	130	0·643	1·3	24·4	1·0	1·27	2·36	1·01	28·7
2	♀	140	0·679	1·44	23·2	1·03	1·34	7·46	1·09	32·1
3	♀	170	0·586	1·08	35·5	0·64	1·54	29·87	1·33	18·8
4	♀	170	0·599	1·12	34·2	0·66	1·54	27·27	1·33	15·8
5	♀	170	0·860	2·32	16·5	1·36	1·54	50·65	1·33	74·5
6	♀	200	0·774	1·88	22·9	0·94	1·73	8·67	1·56	20·5
7	♂	205	0·829	2·15	20·4	1·05	1·76	22·16	1·6	34·4
8	♀	210	0·796	1·99	22·4	0·95	1·79	11·17	1·64	21·4
9	♂	220	0·745	1·74	26·5	0·79	1·85	5·95	1·72	1·16
10	♀	220	0·752	1·77	26·0	0·8	1·85	4·32	1·72	2·91
11	♂	230	0·749	1·76	27·0	0·77	1·91	7·85	1·79	1·68
12	♀	230	0·739	1·71	27·8	0·74	1·91	10·47	1·79	4·47
13	♂	232	0·952	2·84	16·8	1·22	1·92	47·92	1·81	56·9
14	♂	300	0·78	1·91	30·0	0·64	2·3	16·96	2·34	18·4
15	♂	300	0·854	2·28	25·2	0·76	2·3	0·87	2·34	2·56
16	♂	305	1·03	3·34	17·4	1·09	2·33	43·34	2·38	40·3
17	♂	320	0·939	2·77	21·7	0·87	2·41	14·94	2·5	10·8
18	♂	330	0·745	1·74	35·3	0·53	2·47	29·55	2·57	6·62
19	♀	370	1·01	3·22	20·7	0·87	2·67	20·6	2·89	11·4
20	♀	370	0·933	2·73	24·4	0·74	2·67	2·25	2·89	5·54
21	♂	400	0·971	2·96	23·8	0·74	2·83	4·59	3·12	5·16
22	♂	420	0·905	2·57	28·4	0·61	2·93	12·29	3·28	21·6
23	♂	430	1·03	3·32	22·3	0·77	2·98	11·41	3·35	0·89
24	♂	490	1·02	3·24	25·1	0·66	3·26	0·61	3·82	15·2
25	♀	500	0·955	2·87	28·7	0·57	3·31	13·29	3·9	25·4
26	♀	550	0·939	2·77	31·9	0·5	3·54	21·75	4·29	35·4
27	♀	555	1·15	4·15	21·4	0·75	3·57	16·25	4·33	4·16
28	♂	600	1·29	5·22	18·0	0·87	3·77	38·46	4·68	11·5
29	♀	620	1·13	4·03	23·8	0·65	3·86	4·4	4·84	16·7
30	♂	640	1·3	5·33	18·4	0·83	3·95	34·94	4·99	6·81
31	♀	640	1·03	3·36	29·3	0·53	3·95	14·94	4·99	32·6
32	♀	738	1·12	3·92	27·7	0·53	4·37	10·30	5·76	22·9
33	♂	790	1·29	5·22	21·9	0·66	4·58	13·97	6·16	15·6
34	♀	830	1·21	4·57	25·9	0·55	4·75	3·79	6·47	29·4
35	♂	950	1·23	4·71	27·6	0·5	5·22	9·77	7·41	36·6
				Average	24·9	0·78	—	16·44	—	19·68

A. *Mammals.*—In Table I are given the figures and calculations for 35 guinea-pigs ranging in weight from 130 to 950 grm. (*i.e.* increasing more than sevenfold). From this table it is at once evident that, as would be expected, the radius of the aorta increases much more slowly than the weight of the animal. The area of the aortic cross-section also increases more slowly than the body weight (though of course much more rapidly than the radius), so that the ratio of the sectional area of the aorta to the body weight decreases steadily as the weight of the animal increases. But it appears on calculation that the body weight (W) to the nth power (where n is approximately 0·70–0·72) divided by the sectional area (A) is a constant (k).

This gives us the formula $W^n/A = k$, which indicates that the sectional area of the aorta is a simple function of the surface of the body since, as was shown in a previous paper, the body surface, which can be calculated from the formula $S = kW^n$ is more accurately determined by taking n to be approximately 0·71 – 0·72 than by taking it equal to 2/3 as was done by Meeh (5). Now it has been proved on former occasions that the blood volume is proportional to the body surface, hence it follows that the sectional area of the aorta is proportional to the blood volume of the individual.

Table I further shows that the average value of k is 24·9, corresponding to an n of 0·71, which is by calculation the best n for these individuals, and that if the aortic cross-section be calculated from our formula $A = W^n/k$ using these values for n and k, the average percentage deviation between calculated and observed values is 16·44. If, on the other hand, the sectional area is expressed as a percentage of the body weight (0·78), the average deviation between the calculated and the observed values is 19·68 per cent.

It may be stated further that if the value 0·72 is taken for n, the average value of k becomes 26·5, and, if the sectional area is calculated from $A = W^{0 \cdot 72}/26 \cdot 5$, the average percentage deviation between the calculated and the observed figures is found to be 16·53.

In order to bring out the various points more clearly, to get rid of irregularities due to individual variations in the animals, and to diminish the influence of experimental error, the animals have been grouped in Table II.

In this table the guinea-pigs are arranged in five groups according to weight, and the weights, the aortic radii, and the aortic cross-sections of the animals in each group averaged. The other figures are calculated from these average values.

It is found that, under these circumstances, the best n is 0·72 (exactly as we found to be the case in calculating the surface from the body weight), and the average value of k is 25·6. Using these values for n and k, the average deviation between the calculated and the observed figures is 2·97 per cent., whereas, if the sectional area be calculated as a percentage (0·78) of the body weight, the average percentage deviation becomes 14·2, that is to say, nearly five times as large. Moreover, it will be observed that, while the k

Table II.—Guinea-pigs (grouped).

Group.	Numbers of individuals from Table I in group.	Average body weight.	Average radius of aorta.	Average area of aortic cross-section.	$k = W^{0\cdot72}/A$.	Aortic cross-section as percentage of body weight.	Cross-section calculated. $A = W^{0\cdot72}/k$. ($k = 25\cdot6$.)	Difference between cross-section calculated and observed.	Cross-section calculated as percentage (0·78) of body weight.	Difference between cross-section calculated and observed.
		grm.	mm.	sq. mm.				per cent.		per cent.
A	1–5	156	0·679	1·45	26·2	0·93	1·48	2·03	1·22	18·85
B	6–13	218	0·794	1·98	24·4	0·91	1·89	4·76	1·7	16·47
C	14–20	328	0·904	2·57	25·2	0·78	2·55	0·78	2·57	0·0
D	21–27	478	0·998	3·13	27·1	0·65	3·32	5·72	3·73	16·09
E	28–35	726	1·204	4·55	25·2	0·63	4·48	1·56	5·66	19·61
				Average	25·6	0·78	—	2·97	—	14·2

exhibits no periodic variation as the weight of the animal increases, the figure representing the sectional area in percentage of body weight decreases with absolute regularity from 0·93 to 0·63.

As regards the question of sex, if the males and females be considered separately in Table I, it will be seen that the average k for the 18 males is smaller than the k for the 17 females. Thus, with n equal to 0·71, the k for the males is 23·9, and that for the females 26, while with n equal to 0·72, k is 25·4 for the males and 27·6 for the females, indicating in each case that the male animals had somewhat larger aortas than had the females of corresponding weights. But this is a point to which we shall return.

In Table III are given the figures and calculations for the aortas of 27 rats, ranging in weight from 30·1 to 303 grm. (*i.e.* increasing more than tenfold). The average aortic constant (k) is 21·37, with an n of 0·71, which is the best n for these observations, and the average aortic percentage (*i.e.* sectional area of aorta expressed as a percentage of body weight) is 1·27. It is seen that, as in the case of the guinea-pigs, the variations of the aortic constant show no periodicity, but the aortic percentage decreases markedly and steadily, although not regularly, as the animals increase in weight. If the area of the aortic cross-section is calculated by our formula, the average deviation between the calculated and the observed figures is 11·09 per cent., while it is 19·72 per cent., or nearly twice as large, when the area is calculated in per cent. of body weight. If the value 0·72 be taken for n, k becomes 22·36, and, if these values be used in calculating the sectional aortic area by our formula, the average percentage deviation of the observed from the calculated figures is 11·37 per cent.

Table III.—Rats (individuals).

No.	Sex.	Body weight.	Radius of aorta.	Area of aortic cross-section.	$k = W^{0.71}/A$.	Aortic cross-section as percentage of body-weight.	Cross-section calculated. $A = W^{0.71}/k$. ($k = 21·37$.)	Difference between cross-section calculated and observed.	Cross-section calculated as percentage (1·27) of body weight.	Difference between cross-section calculated and observed.
		grm.	mm.	sq. mm.				per cent.		per cent.
1	♀	30·1	0·436	0·597	18·78	1·98	0·525	13·72	0·382	56·28
2	♀	39·1	0·417	0·546	24·74	1·4	0·632	13·61	0·497	9·84
3	♂	40·3	0·468	0·681	20·08	1·7	0·646	6·35	0·512	34·18
4	♂	47·2	0·455	0·657	23·71	1·38	0·722	9·83	0·599	8·68
5	♀	53·0	0·493	0·764	21·94	1·44	0·784	2·56	0·673	13·52
6	♂	56·0	0·510	0·815	21·38	1·46	0·816	0·12	0·711	14·63
7	♀	57·7	0·548	0·941	18·92	1·63	0·833	12·97	0·733	28·37
8	♀	69·7	0·566	1·01	20·16	1·45	0·953	5·98	0·885	14·12
9	♀	75·5	0·599	1·12	19·23	1·48	1·01	1·09	0·959	16·79
10	♀	79·5	0·580	1·06	21·09	1·31	1·05	0·95	1·01	4·95
11	♂	84·5	0·669	1·4	16·67	1·66	1·09	28·44	1·07	30·84
12	♂	96·7	0·484	0·735	34·93	0·76	1·2	38·8	1·23	40·24
13	♂	104·0	0·643	1·3	20·81	1·25	1·27	2·36	1·32	1·52
14	♂	108·0	0·77	1·86	14·94	1·72	1·3	40·38	1·37	35·77
15	♂	140·0	0·669	1·4	23·85	1·0	1·56	10·26	1·78	21·35
16	♂	141·0	0·707	1·57	21·38	1·11	1·57	0·0	1·79	12·29
17	♂	141·0	0·669	1·4	23·97	0·99	1·57	10·83	1·79	21·79
18	♀	141·0	0·678	1·44	23·31	1·02	1·57	8·28	1·79	19·55
19	♂	157·0	0·723	1·64	22·09	1·04	1·69	2·96	1·99	17·59
20	♂	157·6	0·77	1·86	19·54	1·18	1·7	9·41	2·0	7·0
21	♂	160·0	0·819	2·1	17·47	1·31	1·72	22·09	2·03	3·45
22	♂	164·0	0·723	1·64	23·32	1·0	1·75	6·29	2·08	21·15
23	♂	172·0	0·812	2·07	18·67	1·20	1·81	14·36	2·18	5·05
24	♂	175·0	0·732	1·68	23·29	0·96	1·83	8·2	2·22	24·32
25	♂	179·0	0·755	1·79	22·22	1·0	1·86	3·76	2·27	21·15
26	♂	289·0	0·885	2·47	22·62	0·85	2·61	5·36	3·67	32·7
27	♂	303·0	1·01	3·22	17·95	1·06	2·7	17·78	3·85	16·36
				Average	21·37	1·27	—	11·09	—	19·72

In Table IV the rats are arranged in six groups according to weight, and the aortic constant and aortic percentage are calculated from the average figures of these groups. As in the case of the grouped guinea-pigs, the best n is 0·72, giving an average k of 21·9. It will be observed that in these grouped animals the variations in the aortic constants of the groups are very small and are non-periodic, while the aortic percentage falls markedly and very regularly from 1·67 to 0·96. Using the values just stated for n and k, the average deviation between the calculated and the observed figures for the aortic cross-section is only 1·18 per cent., while it is 16·22 per cent. (nearly 14 times as large) if the area be calculated in per cent. of body weight.

Table IV.—Rats (grouped).

Group.	Numbers of individuals from Table III in group.	Average body weight.	Average radius of aorta.	Average area of aortic cross-section.	$k = W^{0\cdot72}/A$.	Aortic cross-section as percentage of body weight.	Cross-section calculated. $A = W^{0\cdot72}/k$. $(k = 21\cdot9.)$	Difference between cross-section calculated and observed.	Cross-section calculated as percentage (1·28) of body weight.	Difference between cross-section calculated and observed.
		grm.	mm.	sq. mm.				per cent.		per cent.
A	1–3	36·5	0·441	0·61	21·9	1·67	0·609	0·16	0·467	30·62
B	4–7	53·5	0·503	0·793	22·1	1·48	0·802	1·12	0·685	15·77
C	8–12	81·2	0·583	1·07	22·2	1·32	1·08	0·93	1·04	2·88
D	13–18	129·0	0·691	1·5	22·1	1·16	1·51	0·66	1·65	9·09
E	19–25	166·4	0·763	1·83	21·7	1·1	1·82	0·55	2·13	14·08
F	26–27	296·0	0·952	2·85	21·1	0·96	2·75	3·64	3·79	24·8
				Average ………	21·9	1·28	—	1·18	—	16·22

Table V.—Rabbits (individuals).

No.	Sex.	Body weight.	Radius of aorta.	Area of aortic cross-section.	$k = W^{0\cdot71}/A$.	Aortic cross-section as percentage of body weight.	Cross-section calculated. $A = W^{0\cdot71}/k$. $(k = 22\cdot49.)$	Difference between cross-section calculated and observed.	Cross-section calculated as percentage (0·609) of body weight.	Difference between cross-section calculated and observed.
		grm.	mm.	sq. mm.				per cent.		per cent.
1	♀	310	0·996	3·12	18·82	1·006	2·61	19·54	1·89	65·08
2	♀	370	0·926	2·7	24·66	0·73	2·96	8·78	2·25	20·0
3	♀	530	1·14	4·1	20·96	0·774	3·82	7·33	3·23	26·93
*4	—	767	1·43	6·44	17·35	0·84	4·97	33·6	4·67	37·9
5	♂	1330	1·41	6·25	26·43	0·47	7·35	14·96	8·1	22·84
6	♂	1420	1·47	6·77	25·56	0·477	7·7	12·08	8·65	21·73
*7	—	1640	1·85	10·23	18·74	0·624	8·52	20·07	9·99	2·4
8	♂	1885	1·59	7·97	26·55	0·423	9·41	15·3	11·48	30·57
9	♂	2080	1·84	10·64	21·32	0·512	10·09	5·45	12·67	16·02
*10	—	2096	2·07	13·45	16·96	0·642	10·15	32·51	12·76	5·41
11	♂	2100	1·71	9·15	24·97	0·436	10·16	9·94	12·79	28·46
12	♂	2550	1·74	9·52	27·54	0·373	11·66	18·35	15·53	38·7
				Average ………	22·49	0·609	—	16·49	—	26·34

* The data for the individuals indicated are taken from Keilson (6).

Table V gives the figures for the aortas of 12 rabbits, ranging in weight from 310 grm. to 2250 grm. (*i.e.* increasing more than eightfold). The average aortic constant (k) is 22·49, with an n (best n) of 0·71, and the average aortic percentage is 0·609. The variations of the aortic constant show no periodicity, but the aortic percentage decreases very greatly (although not regularly) as the animals increase in weight. When the aortic area (*i.e.* area of the cross-section of the aorta) is calculated by our formula, the average deviation of the observed from the calculated figures is 16·49 per cent., while it is 26·34 per cent. if the area be calculated in per cent. of body weight.

If the value 0·72 be taken for n, k becomes 24·15.

Table VI.—Rabbits (grouped).

Group.	Numbers of individuals from Table V in group.	Average body weight.	Average radius of aorta.	Average area of aortic cross-section.	$k = W^{0.71}/A$.	Aortic cross-section as percentage of body weight.	Cross-section calculated. $A = W^{0.71}/k$. ($k = 22 \cdot 1$.)	Difference between cross-section calculated and observed.	Cross-section calculated as percentage (0·628) of body weight.	Difference between cross-section calculated and observed.
		grm.	mm.	sq. mm.				per cent.		per cent.
A	1–2	340	0·961	2·91	21·6	0·856	2·84	2·46	2·14	35·98
B	3–4	649	1·29	5·27	18·7	0·812	4·45	18·43	4·08	29·17
C	5–6	1375	1·44	6·51	26·0	0·473	7·65	14·9	8·64	24·65
D	7–8	1763	1·72	9·1	22·2	0·516	9·13	0·33	11·07	17·71
E	9–12	2207	1·84	10·69	22·1	0·484	10·71	0·19	13·86	22·87
				Average	22·1	0·628	—	7·26	—	26·08

In Table VI the rabbits are arranged in five groups according to weight, and the aortic constant and aortic percentage are calculated from the average figures of these groups. In this case the best n is 0·71, giving an average k of 22·1. The variations in the aortic constant are without periodicity, but the aortic percentage falls gradually, though not quite regularly, from 0·856 in the lightest, to 0·484 in the heaviest group. Using the above values for n and k, the average deviation of the observed figures from the calculated is 7·26 per cent., while it is 26·08 per cent. (between three and four times as great) if the area be calculated as a percentage of body weight.

We now return to examine the question of sex as regards its bearing on the size of the aorta in these mammals. Since the number of our observations within any one of the species can hardly be regarded as sufficient to justify a general inference, we have taken all three species of animals together and made the figures comparable, *inter se*, by reducing them in terms of a common standard. When this is done, and the male and female animals are taken separately, it appears that the sectional area of the aorta in the male expressed as a function of the body surface is about 3 per cent. greater than it is in a female of the same weight. This is of special interest in view of the fact that a difference of the same size and character was observed by two of us in the blood volume of male and female rabbits. It may be noted, further, in this connection that, so far as we have yet ascertained from our data, the heart in the male animal is somewhat larger than in the female of the same body weight and species.

Table VII.—Ducks (individuals).

No.	Sex.	Body weight.	Radius of aorta.	Area of aortic cross-section.	$k = W^{0.70}/A$.	Aortic cross-section as percentage of body weight.	Cross-section calculated. $A = W^{0.70}/k$. ($k = 10·75$.)	Difference between cross-section calculated and observed.	Cross-section calculated as percentage (1·56) of body weight.	Difference between cross-section calculated and observed.
		grm.	mm.	sq. mm.				per cent.		per cent.
1	—	70	0·764	1·84	10·66	2·62	1·82	0·82	1·09	68·04
2	—	70	0·758	1·8	10·87	2·57	1·82	1·1	1·09	64·84
3	—	70	0·745	1·74	11·21	2·49	1·82	4·4	1·09	59·34
4	—	520	1·56	7·64	10·42	1·47	7·38	3·52	8·11	5·8
5	—	600	1·66	8·6	10·23	1·43	8·25	4·24	9·36	8·12
6	♀	1180	1·99	12·42	11·39	1·05	13·14	5·48	18·41	32·45
7	♂	1330	2·14	14·3	10·75	1·08	14·3	0·0	20·75	31·09
8	♀	1420	2·16	14·72	10·93	1·04	14·96	1·6	22·15	33·54
9	♀	2070	2·47	19·15	10·91	6·93	19·48	1·69	32·29	40·69
10	♀	2850	2·86	25·8	10·16	0·91	24·37	5·87	44·46	41·97
		Average			10·75	1·56	—	2·87	—	38·59

B. *Birds.*—Table VII gives the figures for the aortas of 10 ducks, ranging in weight from 70 grm. to 2850 grm. (*i.e.* increasing more than fortyfold). The average aortic constant (k) is 10·75 with an n (best n) of 0·70, and the average aortic percentage is 1·56. The variations of the aortic constant are small and show no periodicity, but the aortic percentage decreases very greatly and with absolute regularity as the animals increase in weight. When the aortic area (A) is calculated by our formula, the average deviation of the observed from the calculated figures is only 2·87 per cent., while it is 38·59 per cent. (more than 13 times as great) if the area be calculated in per cent. of body weight.

If the value 0·71 be taken for n, k becomes 11·54 and the average deviation between calculated and observed values is 3·12 per cent.; with n equal to 0·72, k is 12·19. That the value 0·71 for n is very nearly as good as the "best" n (0·70) is shown by the fact that while the average deviations per cent. are 2·87 and 3·12 respectively with $n = 0·70$ and $n = 0·71$, the *mean* *deviations* calculated by the method of least squares are 3·67 and 3·63 per cent. respectively with the same values of n. That is to say that the value 0·71 gives a very slightly smaller *mean* deviation than the value 0·70.

Table VIII.—Fowls (individuals).

No.	Sex.	Body weight.	Radius of aorta.	Area of aortic cross-section.	$k = W^{0.72}/A$.	Aortic cross-section as percentage of body weight.	Cross-section calculated. $A = W^{0.72}/k$. ($k = 21·21$.)	Difference between cross-section calculated and observed.	Cross-section calculated as percentage (1·14) of body weight.	Difference between cross-section calculated and observed.
		grm.	mm.	sq. mm.				per cent.		per cent.
1	—	40·6	0·462	0·669	21·51	1·648	0·679	1·47	0·463	54·13
2	—	42·7	0·462	0·669	22·31	1·567	0·704	4·97	0·487	43·53
3	—	43·9	0·484	0·736	20·54	1·677	0·718	2·51	0·5	47·2
4	♀	919	1·45	6·6	20·61	0·718	6·41	2·96	10·48	37·02
5	♀	1580	1·72	9·3	21·61	0·589	9·48	1·9	18·01	48·31
6	♀	1598	1·77	9·81	20·65	0·614	9·55	2·72	18·22	46·16
			Average		21·21	1·14	—	2·76	—	46·06

In Table VIII are given the figures for the aortas of six fowls, ranging in weight from 40·6 grm. to 1598 grm. (*i.e.* increasing nearly fortyfold). The average aortic constant (k) is 21·21 with an n (best n) of 0·72, and the average aortic percentage is 1·14. The variations of the aortic constant are quite small and show no periodicity, but the aortic percentage decreases very greatly and almost regularly from 1·648 in the lightest animal to 0·614 in the heaviest. When the aortic area (A) is calculated by our formula the average deviation of the observed from the calculated figures is only 2·76 per cent., while it is 46·06 per cent. (nearly 17 times as great) if the area be calculated in percentage of body weight.

With n taken as 0·71, k becomes 20·08 and the average percentage deviation between observed and calculated figures is 3·28.

Table IX contains the figures for 10 ptarmigan purchased from a game-dealer. These were birds which had been shot, and they show the greatest range of weight we were able to obtain, namely, from 470 grm. to 710 grm. The average aortic constant (k) is 11·4 with an n (best n) of 0·71, and the average aortic percentage is 1·4. The variations of the aortic constant show no periodicity, but the aortic percentage decreases from 1·66 in the lightest to 1·23 in the heaviest animal. When the aortic area (A) is calculated by our formula the average deviation of the observed from the calculated figures is 7·12 per cent., while if the area be calculated as a percentage of body weight it is 7·22. If the value 0·72 be taken for n, k becomes 12·2.

Table IX.—Ptarmigan (individuals).

No.	Sex.	Body weight.	Radius of aorta.	Area of aortic cross-section.	$k = W^{0.71}/A$.	Aortic cross-section as percentage of body weight.	Cross-section calculated. $A = W^{0.71}/k$. ($k = 11·4$.)	Difference between cross-section calculated and observed.	Cross-section calculated as percentage (1·40) of body weight.	Difference between cross-section calculated and observed.
		grms.	mm.	sq. mm.				per cent.		per cent.
1	♀	470	1·58	7·8	10·1	1·66	6·92	12·71	6·58	18·54
2	♀	470	1·4	6·19	12·8	1·32	6·92	10·53	6·58	5·93
3	♀	530	1·48	6·84	12·6	1·29	7·54	9·28	7·42	7·82
4	♀	538	1·59	7·93	10·9	1·47	7·62	4·06	7·53	5·31
5	♀	553	1·51	7·19	12·3	1·3	7·77	7·46	7·74	7·11
6	♀	590	1·63	8·35	11·1	1·42	8·14	2·58	8·26	1·0
7	♀	600	1·68	8·91	10·5	1·49	8·23	8·26	8·4	6·07
8	♀	630	1·65	8·58	11·3	1·36	8·52	0·7	8·82	2·72
9	♀	650	1·75	9·58	10·4	1·47	8·72	9·87	9·1	5·27
10	♀	710	1·67	8·71	12·1	1·23	9·24	5·73	9·94	12·37
				Average	11·4	1·4	—	7·12	—	7·22

Table X.—Ptarmigan (grouped).

Group.	Numbers of individuals, from Table IX in group.	Average body weight.	Average radius of aorta.	Average area of aortic cross-section.	$k = W^{0.72}/A$.	Aortic cross-section as percentage of body-weight.	Cross-section calculated. $A = W^{0.72}/k$. ($k = 12·1$.)	Difference between cross-section calculated and observed.	Cross-section calculated as percentage (1·41) of body weight.	Difference between cross-section calculated and observed.
		grm.	mm.	sq. mm.				per cent.		per cent.
A	1–2	470	1·49	6·69	12·0	1·49	6·94	0·72	6·63	5·43
B	3–4	534	1·53	7·39	12·5	1·38	7·6	2·76	7·53	1·86
C	5–7	581	1·61	8·15	12·0	1·4	8·08	0·87	8·19	0·49
D	10–12	663	1·69	8·96	12·0	1·35	8·89	0·78	9·35	4·17
				Average	12·1	1·41	—	1·28	—	2·99

In Table X the ptarmigan are arranged in four groups according to weight, and the aortic constant and aortic percentage are calculated from the average figures of these groups. In this case the best n is 0·72, giving an average value for k of 12·1. The variations in the aortic constant are small and non-periodic, but the aortic percentage falls gradually though not quite regularly from 1·49 to 1·35. Using the above values for *n* and *k*, the average deviation of the observed figures from the calculated is 1·28 per cent., while it is 2·99 per cent. (more than twice as great) if the area be calculated as a percentage of the body weight.

Table XI.—Sparrows (individuals).

No.	Body weight.	Radius of aorta.	Area of aortic cross-section.	$k = W^{0.71}/A$.	Aortic cross-section as percentage of body weight.	Cross-section calculated. $A = W^{0.71}/k$. ($k = 22·6$.)	Difference between cross-section calculated and observed.	Cross-section calculated as percentage (1·771) of body weight.	Difference between cross-section calculated and observed.
	grm.	mm.	sq. mm.				per cent.		per cent.
1	22·05	0·35	0·385	23·1	1·743	0·398	3·26	0·391	1·53
2	22·55	0·366	0·42	21·8	1·782	0·405	3·7	0·399	5·26
3	23·1	0·398	0·487	19·1	2·105	0·412	15·77	0·409	19·03
4	24·6	0·414	0·537	18·1	2·098	0·429	25·18	0·436	23·2
5	26·55	0·35	0·385	26·6	1·45	0·453	15·01	0·47	18·09
6	26·6	0·35	0·385	26·7	1·448	0·454	15·2	0·471	18·3
			Average	22·6	1·771	—	13·02	—	14·24

Table XI gives the figures for the aortas of six sparrows ranging in weight from 22·05 grm. to 26·6 grm., a very small range indeed, but the best which we were able to obtain at the time. The average aortic constant (*k*) is 22·6 with an *n* (best *n*) of 0·71, and the average aortic percentage is 1·771. The variations of the aortic constant show no periodicity. When the aortic area (A) is calculated by our formula the average percentage deviation between the calculated and the observed figures is 13·02, and it is 14·24 if the area be calculated as a percentage of body weight.

If *n* be taken as 0·70 the value of *k* is 21·9, and with an *n* of 0·72 *k* is 23·4.

C. *Thoma's Observations on Man.*—Table XII contains the figures for the aortas of 33 human individuals calculated from the grouped observations published by R. Thoma (7). The individuals in question ranged in *age* from two months to 29 years and the range in weight of the groups is from 8941 grm. to 49,000 grm. The best *n* is 0·70, which gives an average value for *k* of 5·03. The variations in the aortic constant are without periodicity, but the aortic percentage falls gradually though not quite regularly from 1·309 in the lightest group to 0·804 in the heaviest. Using these values for *n* and *k*, the average percentage deviation of the observed from the

Table XII.—Man (grouped). Thoma's Observations.*

Group.	Number of individuals in group.	Average body weight.	Average radius of aorta.	Average area of aortic cross-section.	$k = W^{0.70}/A$.	Aortic cross-section as percentage of body weight.	Cross-section calculated. $A = W^{0.70}/k$. ($k = 5·03$.)	Difference between cross-section calculated and observed.	Cross-section calculated as percentage (1·052) of body weight.	Difference between cross-section calculated and observed.
		grms.	mm.	sq. mm.				per cent.		per cent.
A	7	8941	6·1	**117·0**	**4·99**	**1·309**	**116·0**	**0·86**	**94·1**	**24·34**
B	9	11950	6·7	**141·0**	**5·07**	**1·18**	**142·1**	**0·77**	**125·7**	**12·17**
C	5	13630	7·25	**165·0**	**4·75**	**1·211**	**155·8**	**5·91**	**143·4**	**15·06**
D	3	17510	7·65	**184·0**	**5·08**	**1·051**	**185·7**	**0·92**	**184·2**	**0·11**
E	4	43250	10·2	**326·0**	**5·4**	**0·754**	**349·6**	**6·75**	**455·0**	**28·35**
F	5	49000	11·2	**394·0**	**4·87**	**0·804**	**381·5**	**3·28**	**515·5**	**23·57**
				Average	**5·03**	**1·052**	—	**3·08**	—	**17·27**

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* Thoma's data are printed in light type. The figures calculated by us are printed in heavy type.

calculated figures is 3·08, while it is 17·27 (more than five and a-half times as great) if the area be calculated as a percentage of the body weight.

If the value 0·71 be taken for n the value of k becomes 5·55, and with an n of 0·72 k is 6·13. Taking n as 0·71 the average percentage deviation of the observed values from those calculated by our formula is 3·16 as compared with the deviation of 3·08 per cent. with n equal to 0·70. Or if the appropriate allowance be made for the number of individuals in each of the groups, the figures for the average percentage deviation of the observed values from the theoretical values given by our formula are 2·69 with an n of 0·70 and 2·73 with n of 0·71, indicating the fact that the value 0·71 for n is only very slightly less good in these observations than the value 0·70.

Thoma himself, who, in his great monograph (7) on the size and weight of the various parts of the human body under normal and diseased conditions, endeavoured to establish a definite quantitative relation between the body weight and the aortic radius, defined the correlation which he found to exist in the statement that the body weight divided by the cube of the aortic radius was approximately constant over a wide range of weight.

This he expressed in the formula $W/r^3 = K$. The formula was purely empirical, and possessed no special biological significance, though it represented the experimental data in an extremely satisfactory manner. It will be seen, however, that it is readily transformable into the formula $W^{\frac{2}{3}}/\pi r^2 = k$, which is the formula deduced by us from our observations upon animals ($W^n/A = k$) if one gives n the value $\frac{2}{3}$ or 0·67 instead of 0·70–0·72, as we have found it to be. This latter formula is a rational formula, since it indicates, as has already been pointed out, that the aortic area (like the blood volume) is a function of the body surface.

Table XIII.—Man (grouped). Thoma's Observations.*

Group.	Number of individuals in group.	Average body weight.	Average radius of aorta.	$K_1 = W/r^{n_1}$ ($n_1 = 3$).	$K_2 = W/r^{n_2}$ ($n_2 = 2·82$).	Aortic radius calculated. $r_1 = \sqrt[n_1]{(W/K_1)}$. ($n_1 = 3$, $K_1 = 38·3$.)	Difference between aortic radius calculated and observed.	Aortic radius calculated. $r_2 = \sqrt[n_2]{(W/K_2)}$. ($n_2 = 2·82$, $K_2 = 55·8$.)	Difference between aortic radius calculated and observed.
		grm.	mm.				per cent.		per cent.
A	7	8,941	6·1	**39·4**	**54·6**	**6·16**	**0·97**	**6·05**	**0·83**
B	9	11,950	6·7	**39·7**	**56·0**	**6·78**	**1·18**	**6·71**	**0·15**
C	5	13,630	7·25	**35·8**	**51·8**	**7·09**	**2·26**	**7·03**	**3·13**
D	3	17,510	7·65	**39·1**	**56·4**	**7·70**	**0·65**	**7·68**	**0·39**
E	4	43,250	10·2	**40·8**	**61·9**	**10·41**	**2·02**	**10·58**	**3·59**
F	5	49,000	11·2	**34·9**	**53·9**	**10·86**	**3·13**	**11·06**	**1·27**
		Average		**38·3**	**55·8**	—	**1·70**	—	**1·56**

* Thoma's data are printed in light type. The figures calculated by us are printed in heavy type.

In Table XIII we have calculated K for Thoma's figures from the formula $W/r^n = K$, first giving n the value 3 as in Thoma's formula, and then giving it the value 2·82, which corresponds to $n = 0·71$ in our formula, and is the best n for Thoma's observations. It then appears that if the aortic radius be calculated by means of these values of K the average percentage deviation between r calculated and r observed is 1·70 when n has the value 3, while it is only 1·56 when n has the value 2·82. Moreover, if the appropriate allowance be made for the number of individuals in each group the figures become 1·65 with n taken as 3, and 1·35 with n taken as 2·82. It will, of course, readily be apprehended that differences of this amount in the radius assume a considerable importance when calculations are made by area (*i.e.* πr^2), as is necessary in referring the aortic area to the body surface.

In Table XIV are tabulated our main results arranged in such a manner as to show at a glance the range of weight, the best n, the value of k, the percentage deviation, and so forth for each species of animal. It will be seen from the averages brought out at the foot of the table that taking all

Table XIV.—Constants, Average Deviations, etc.

Animal.	Range of weight.	Weight of heaviest in terms of lightest.	Best n.	Aortic constant (k). $k = W^n/A$.			Average percentage deviation.				Deviation by weight divided by deviation by best n.
				$n = 0·70$.	$n = 0·71$.	$n = 0·72$.	$n = 0·70$.	$n = 0·71$.	$n = 0·72$.	As percentage of body weight.	
	grm.										
Guinea-pig : individual …	130–950	7·31	0·71	—	**24·9**	26·5	—	**16·44**	16·53	19·68	1·2
” grouped ……	156–726	4·65	0·72	—	24·2	**25·6**	—	3·33	**2·97**	14·20	4·78
Rat : individual …………	30·1–303	10·1	0·71	—	**21·4**	22·4	—	**11·09**	11·37	19·72	1·78
” grouped ……………	36·5–296	8·11	0·72	—	20·9	**21·9**	—	—	**1·18**	16·22	13·8
Rabbit (tame) : individual	310–2550	8·22	0·71	—	**22·5**	24·2	—	**16·49**	—	26·34	1·6
” grouped …	340–2207	6·5	0·71	—	**22·1**	23·7	—	**7·26**	—	26·08	3·59
Duck : individual ………	70–2850	40·7	0·70	**10·8**	11·5	12·2	**2·87**	3·12	—	38·59	13·5
Fowl : individual…………	40·6–1598	39·4	0·72	—	20·1	**21·2**	—	3·28	**2·76**	46·06	16·7
Ptarmigan : individual …	470–710	1·51	0·71	—	**11·4**	12·2	—	**7·12**	—	7·22	1·01
” grouped ……	470–663	1·41	0·72	—	—	**12·1**	—	—	**1·28**	2·99	2·34
Sparrow : individual ……	22·05–26·6	1·21	0·71	21·9	**22·6**	23·4	—	**13·02**	—	14·24	1·09
Man : grouped……………	8941–49000	5·48	0·70	**5·03**	5·55	6·13	**3·08**	3·16	—	17·27	5·61
Average percentage deviation taking best n { individual …………								9·97		24·55	
grouped ……………								3·15		15·35	

our animals together the average percentage deviation for the individual animals between the calculated and the observed figures for the aortic area is 9·97 when the calculation is made in terms of the body surface, while it is 24·55 (two and a-half times as great) when the area is expressed in percentage of body weight. The corresponding figures for the grouped animals are 3·15 and 15·35 per cent. respectively, a deviation nearly five times as large.

Attention may further be drawn to the fact that although the technique of these aortic measurements is in the nature of the case much less exact than that employed by two of us in measuring the blood volume, and therefore gives much larger figures for the percentage deviation, yet this deviation is found to be reduced to precisely the same extent in both cases by grouping the animals. Thus in the present instance the ratio between individual and grouped percentage deviations (of course reckoning by the body surface) is 9·97/3·15, *i.e.* 3·2, while in the case of the blood volume experiments it was 4·43/1·39, or 3·2 again. From the table it is also clearly seen that the greater the range of weight of the animal observed the more misleading and erroneous is the result obtained by calculating the aortic area in percentage of the body weight. Thus, while in the case of the ptarmigan and sparrow, where we have only a small range of weight, the difference is comparatively small, in the case of ducks, fowls, and rats, which show the widest range, the difference may properly be spoken of as enormous, being from 13·5 to 16·7 times as large. This shows that if one were to attempt to calculate the sectional area of the aorta of a small animal, for example, from the ascertained aortic area of a large one of the same species, expressed as a percentage of body weight, the result would inevitably be grossly misleading, while if it were calculated by our formula a true estimate would be obtained, correct within the limits of the experimental errors.

In Table XV are given the mean deviations as calculated by the method of least squares for the eight species concerned in these observations, both individually and grouped. From the averages of these it can be seen that if one were to make only a single observation and this differed by **30** per cent. from the theoretical value given by our formula it would be probable that the aortic area in question was abnormal in size, while if it were expressed as a percentage of body weight it would have to differ from the theoretical area by at least **60** per cent. before one could say with the same degree of certainty that it was abnormal.

If, however, a series of observations were made and averaged, it follows from our figures for the mean deviation for grouped animals that if the difference between this average and the theoretical value given by our

Table XV.—Mean Deviations.

Species.	Best n.	Mean deviation using best n in the formula $A = W^n/k$.	Mean deviation when aortic area is calculated in percentage of body weight.
Guinea-pig: individual	0·71	21·51	25·76
,, grouped	0·72	3·91	17·81
Rat: individual	0·71	15·75	23·54
,, grouped	0·72	1·8	20·45
Rabbit (tame): individual	0·71	19·47	32·08
,, grouped	0·71	11·92	29·96
Duck: individual	0·70	3·67	45·93
Fowl: individual	0·72	3·25	50·7
Ptarmigan: individual	0·71	8·41	9·1
,, grouped	0·72	1·78	4·11
Sparrow: individual	0·71	16·51	17·84
Man: grouped	0·70	4·32	21·57
Average mean deviation taking best n.	individuals	14·21	29·27
	grouped	**4·75**	**18·78**

formula was as much as **10** per cent., the aortic area would probably be abnormal, and if it amounted to **15** per cent. it would be almost certain that the aortic area was abnormally large or small. But if the measurements were expressed in percentage of body weight it would only be possible to say with the same degree of certainty that the aortic area of an animal was abnormal when it differed from the calculated value by **60** per cent.

Conclusion.

Within a wide range of weight in any given species of warm-blooded animal the sectional area of the lumen of the aorta is proportional to the body surface, and can be calculated from the body weight by means of the formula $A = W^n/k$, where n has the value 0·70–0·72 and k is a constant to be ascertained for each particular species.

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The Size of the Trachea in Warm-Blooded Animals, and its Relationship to the Weight, the Surface Area, the Blood Volume, and the Size of the Aorta.

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The analysis of data collected in connection with the investigation of a number of problems in immunity has led to a series of results, in part already published, bearing upon the blood and circulation. The conclusion was reached that in certain cases a precise and definite relationship to the body surface exists in warm-blooded animals in accordance with the formula $W^n/a = k$, where W is the body weight of the animal, a represents the mass of the body fluid, tissue, or organ under investigation, k is a constant, and the value of n is approximately 0·70–0·72.

In view of the fact that the carriage of oxygen is one of the chief functions of the circulation, and that the volume of the blood (1), (2), and the aortic area (3), (4), (area of cross-section of aorta), have been shown by us to be proportional to the body surface in warm-blooded animals, while, as we have also found, the total oxygen capacity is the main factor in determining the size of the heart (5), it appeared to be of interest to examine the size of the channel by which the oxygen gains access to the lungs.

Accordingly, the trachea was measured in two species of mammal and one bird, namely, guinea-pig, rabbit, and ptarmigan. The animals used were healthy individuals in good condition, not previously experimented upon, and many of them were made use of at the same time for measuring the size of the aorta. The guinea-pigs and rabbits had all been kept in the laboratory under as equable conditions as possible as regards food, etc. Pregnant animals were, of course, excluded. The ptarmigan were purchased from a game dealer, and were carefully selected.

Methods.—The technique employed was identical with that described in a previous paper (4) for the determination of the size of the aorta. The *internal* πr of the trachea was carefully measured at a point just above its bifurcation, and from this the radius and the sectional area were determined. As we have pointed out before (4), the method made use of is open to considerable experimental error, but this is greatly reduced by multiplying the number of individual observations of each trachea, and also diminishes greatly with increasing practice. The body weights recorded are the natural weights (in grammes) of the animals immediately before they were killed, and consequently include the weight of the contents of the alimentary canal.

Table I.—Guinea-pig (individuals).

No.	Sex.	Body weight.	Radius of trachea.	Area of tracheal cross-section.	$k = W^{0.72}/T$.	Tracheal cross-section as percentage of body weight.	Cross section calculated. $T = W^{0.72}/k$. ($k = 23\cdot4$.)	Difference between cross-section calculated and observed.	Cross-section calculated as percentage (0·908) of body weight.	Difference between cross-section calculated and observed.
		grm.	mm.	sq. mm.				per cent.		per cent.
1	♂	70	0·605	1·06	20·1	1·52	0·91	16·5	0·635	67·0
2	♀	170	0·814	2·46	16·4	1·45	1·72	43·0	1·54	59·8
3	♂	220	0·745	1·74	28·0	0·791	2·08	16·3	2·0	13·0
4	♀	220	0·892	2·5	19·4	1·14	2·08	20·2	2·0	25·0
5	♂	230	0·742	1·73	29·0	0·753	2·15	19·5	2·19	21·0
6	♀	230	0·733	1·68	29·9	0·73	2·15	21·8	2·19	23·3
7	♂	300	0·796	1·99	30·4	0·664	2·58	22·8	2·72	26·8
8	♂	330	1·035	3·36	19·4	1·02	2·78	15·1	3·0	12·0
9	♀	360	1·031	3·34	20·7	0·928	2·96	12·8	3·27	2·14
10	♀	390	1·082	3·68	19·9	0·944	3·13	17·6	3·54	3·96
11	♀	410	1·157	4·2	18·1	1·02	3·24	29·6	3·72	12·9
12	♂	420	0·97	3·0	25·8	0·714	3·31	9·36	3·81	21·2
13	♂	490	0·988	3·06	28·3	0·625	3·79	17·3	4·45	31·2
14	♂	493	1·179	4·35	20·0	0·883	3·72	16·9	4·48	29·0
15	♀	620	1·115	3·90	26·2	0·629	4·37	10·7	5·63	30·8
16	♀	640	1·203	4·55	23·0	0·711	4·47	1·79	5·81	21·7
				Average	23·4	0·908	—	18·2	—	25·1

Observations.—In Table I are given the figures and calculations for 16 guinea-pigs, ranging in weight from 70 to 640 grm. (*i.e.* increasing more than ninefold). From this table it is seen that, as would be expected, the tracheal area (sectional area of the trachea) increases much more slowly than the body weight, so that the *ratio* of the tracheal area to the body weight decreases steadily as the weight of the animal increases. But it appears on calculation that the body weight (W) to the nth power (where n is approximately 0·70–0·72) divided by the sectional area (T) is a constant (k).

This gives the formula $W^n/T = k$, which indicates that *the tracheal area (area of the cross-section of the trachea) is a simple function of the surface of the body*, since, as we have shown, the body surface may be determined accurately from the formula $S = k \, . \, W^n$ by taking n to be approximately 0·70–0·72 instead of $\frac{2}{3}$, as was done by Meeh (6).

Table I further shows that the average value of k is 23·4, corresponding to an n of 0·72, which is by calculation the best n for these observations, and that if the tracheal area be calculated from the formula $T = W^n/k$, using these values for n and k, the average percentage deviation of the observed from the calculated values is 18·2. If, on the other hand, the sectional area is expressed in percentage of body weight (0·908), the average deviation between the calculated and the observed values is 25·1 per cent.

It may be stated further that if the value 0·71 is taken for n, the average value of k becomes 21·5.

To bring out the various points more clearly, as well as to get rid of irregularities due to individual variations in the animals and to experimental errors, the animals have been grouped in Table II.

The guinea-pigs are arranged in five groups according to weight, and the

Table II.—Guinea-pig (grouped).

Group.	Numbers of individuals from Table I in group.	Average body weight.	Average radius of trachea.	Average area of tracheal cross-section.	$k = W^{0.71}/T$.	Tracheal cross-section as percentage of body weight.	Cross-section calculated. $T = W^{0.71}/k$. ($k = 21 \cdot 3$.)	Difference between cross-section calculated and observed.	Cross-section calculated as percentage (0·965) of body weight.	Difference between cross-section calculated and observed.
		grms.	mm.	sq. mm.				per cent.		per cent.
A	1	70	0·605	1·06	19·3	1·51	0·959	10·53	0·676	56·8
B	2–6	214	0·785	2·02	22·4	0·944	2·12	4·72	2·07	2·42
C	7–10	345	0·986	3·09	20·5	0·896	2·98	3·69	3·33	6·31
D	11–14	453	1·074	3·65	21·1	0·806	3·61	1·11	4·37	16·48
E	15–16	630	1·159	4·23	23·0	0·671	4·56	7·24	6·08	30·43
				Average	21·3	0·965	—	5·46	—	22·49

weights, the tracheal radii, and the tracheal areas of the animals in each group averaged. The other figures are calculated from these average values.

It is found that, under these circumstances, the best n is 0·71, and the average value of k is 21·3. Using these values for n and k, the average deviation between the calculated and the observed figures is 5·46 per cent., while it is 22·49 per cent. if the tracheal area (T) be calculated as a percentage (0·965) of body weight. If the value of n be taken as 0·70, k is 20·1, but, if n be taken as 0·72, k becomes 22·5, and the corresponding average percentage deviation is 5·76 instead of 5·46. If, however, the appropriate allowance be made for the number of individuals in each group, the percentage deviations become 4·22 (with $n = 0·72$) and 4·24 (with $n = 0·71$). So that the value 0·72 for n is in reality slightly better than 0·71, as it was in the case of the individual animals.

Further, it will be observed that, while the k exhibits no periodic variation as the weight of the animal increases, the tracheal percentage (tracheal area expressed in per cent. of body weight) decreases very greatly and with absolute regularity from 1·51 to 0·671.

Table III.—Rabbit (individuals).

No.	Sex.	Body weight.	Radius of trachea.	Area of tracheal cross-section.	$k = W^{0.70}/T$.	Tracheal cross-section as percentage of body weight.	Cross-section calculated. $T = W^{0.70}/k$. ($k = 12·5$.)	Difference between cross-section calculated and observed.	Cross-section calculated as percentage (0·83) of body weight.	Difference between cross-section calculated and observed.
		grm.	mm.	sq. mm.				per cent.		per cent.
1	♀	940	1·8	10·2	11·8	1·084	9·62	6·02	8·2	24·4
2	♀	970	1·76	9·69	12·8	1·001	9·92	2·32	8·47	14·4
3	♀	1100	1·88	11·1	12·2	1·009	10·8	2·78	9·6	15·62
4	♂	1330	1·96	12·06	12·8	0·906	12·3	1·95	11·62	3·79
5	♂	1420	2·09	13·8	11·6	0·972	12·8	7·82	12·4	11·3
6	♀	1600	2·23	15·6	11·2	0·975	14·0	11·4	13·97	11·67
7	♀	1880	2·1	14·0	14·0	0·745	15·7	10·8	16·41	14·7
8	♀	2040	2·45	18·9	11·0	0·927	16·4	13·9	17·82	6·06
9	♂	2080	2·22	15·4	13·6	0·74	16·8	8·34	18·15	14·6
10	♂	2180	2·26	16·1	13·1	0·739	17·4	7·47	19·03	15·39
11	♀	2280	2·42	18·4	12·1	0·807	17·9	2·79	19·9	7·54
12	♀	2750	2·58	20·8	12·3	0·76	20·4	2·45	24·0	12·9
13	♂	2970	2·54	20·3	13·3	0·684	21·5	5·58	25·92	21·68
				Average	12·5	0·873	—	6·43	—	13·39

Table III gives the figures and calculations for the tracheas of 13 rabbits (tame) ranging in weight from 940 grm. to 2970 grm. (*i.e.* increasing more than threefold). The average tracheal constant (*k*) is 12·5 with an *n* of 0·70, which is the best *n* for these observations, and the average tracheal percentage is 0·873. It is seen that, as was the case in the guinea-pigs, the variations of the tracheal constant show no periodicity, but the tracheal percentage falls markedly, though not quite regularly (from 1·084 to 0·684). If the tracheal area (T) is calculated by our formula, the average deviation between the calculated and the observed figures is 6·43 per cent., while it is 13·39 per cent. (more than twice as great) when the area is calculated in per cent. of body weight. If the value 0·71 be taken for *n*, the value of *k* becomes 13·4.

Table IV.—Rabbit (grouped).

Group.	Numbers of individuals from Table III in group.	Average body weight.	Average radius of trachea.	Average area of tracheal cross-section.	$k = W^{0.70}/T$.	Tracheal cross-section as percentage of body-weight.	Cross-section calculated. $T = W^{0.70}/k$. ($k = 12·5$.)	Difference between cross-section calculated and observed.	Cross-section calculated as percentage (0·880) of body-weight.	Difference between cross-section calculated and observed.
		grm.	mm.	sq. mm.				per cent.		per cent.
A	1—2	955	1·78	9·95	12·3	1·04	9·75	2·05	8·4	18·45
B	3—4	1215	1·92	11·58	12·5	0·953	11·54	0·35	10·69	8·33
C	5—7	1633	2·14	14·47	12·3	0·886	14·2	1·9	14·37	0·7
D	8—11	2145	2·34	17·2	12·5	0·802	17·18	0·12	18·88	8·9
E	12—13	2860	2·56	20·6	12·8	0·72	21·02	2·0	25·17	18·16
				Average	12·5	0·88	—	1·28	—	10·91

In Table IV the rabbits are arranged in the usual way in five groups. As in the case of the individual observations the best *n* is 0·70, giving an average *k* of 12·5. The variations in the tracheal constants (for the groups) are small and are non-periodic, while the tracheal percentage falls with absolute regularity from 1·04 in the lightest group to 0·72 in the heaviest group. Using the values just stated for *n* and *k*, the average percentage deviation of the observed from the calculated figures is 1·28, while it is 10·91 (more than eight and a-half times as large) if the area be calculated as a percentage of the body weight. If *n* is taken as 0·71, *k* becomes 13·4.

Table V gives the figures for the tracheas of 10 ptarmigan purchased from a game-dealer. They had been shot for the market, and exhibit the greatest range of weight that we were able to obtain, namely, from 460 grm. to 710 grm. The average tracheal constant (*k*) is 7·45 with an *n* (best *n*) of 0·71, and the average tracheal percentage is 2·16. The variations of the

Table V.—Ptarmigan (individuals).

No.	Sex.	Body weight.	Radius of trachea.	Area of tracheal cross-section.	$k = W^{0.71}/T$.	Tracheal cross-section as percentage of body-weight.	Cross-section calculated. $T = W^{0.71}/k$. ($k = 7·45$.)	Difference between cross-section calculated and observed.	Cross-section calculated as percentage (2·16) of body weight.	Difference between cross-section calculated and observed.
		grms.	mm.	sq. mm.				per cent.		per cent.
1	♀	460	1·81	10·33	7·6	2·25	10·54	1·99	9·94	3·92
2	♀	470	1·84	10·58	7·48	2·25	10·61	0·28	10·14	4·34
3	♀	500	1·81	10·33	7·98	2·06	11·09	6·85	10·8	4·35
4	♀	530	1·97	12·25	7·05	2·31	11·59	5·74	11·43	7·17
5	♀	538	1·96	12·12	7·15	2·27	11·62	4·3	11·62	4·3
5	♀	553	1·95	11·98	7·38	2·17	11·88	0·84	11·94	0·34
7	♀	590	2·01	12·63	7·33	2·14	12·42	1·69	12·73	0·79
8	♀	600	1·99	12·44	7·53	2·08	12·59	1·19	12·96	4·01
9	♀	630	2·04	13·03	7·46	2·07	13·05	0·15	13·6	4·19
10	♀	710	2·11	14·0	7·53	1·97	14·16	1·13	15·23	8·62
				Average	7·45	2·16	—	2·42	—	4·2

tracheal constant show no periodicity, but the tracheal percentage decreases steadily, although not regularly, from 2·25 in the lightest, to 1·97 in the heaviest animal. When the tracheal area (T) is calculated by our formula the average deviation of the observed from the calculated values is 2·42 per cent., while it is 4·2 per cent. (nearly twice as great) if the area be calculated in per cent. of body weight. If the value of *n* be taken as 0·70 *k* becomes 7·0, and if *n* be 0·72 *k* is 7·93.

Table VI.—Ptarmigan (grouped).

Group.	Numbers of individuals from Table V in group.	Average body weight.	Average radius of trachea.	Average area of tracheal cross-section.	$k = W^{0.71}/T$.	Tracheal cross-section as percentage of body-weight.	Cross-section calculated. $T = W^{0.71}/k$. ($k = 7·44$.)	Difference between cross-section calculated and observed.	Cross-section calculated as percentage (2·15) of body weight.	Difference between cross-section calculated and observed.
		grm.	mm.	sq. mm.				per cent.		per cent.
A	1–2	465	1·83	10·46	7·5	2·25	10·53	0·66	10·0	4·6
B	3–4	515	1·89	11·29	7·46	2·19	11·32	0·27	11·07	1·99
C	5–6	547	1·96	12·05	7·3	2·2	11·81	2·03	11·76	2·47
D	7–8	595	2·0	12·54	7·44	2·11	12·54	0·0	12·79	1·95
E	9–10	670	2·08	13·52	7·51	2·02	13·64	0·88	14·41	6·18
				Average	7·44	2·15	—	0·77	—	3·44

In Table VI the ptarmigan are arranged in five groups in the usual manner. In this case, as in that of the individual observations, the best *n* is 0·71, giving an average value for *k* of 7·44. The variations of the tracheal constant are without periodicity, but the tracheal percentage falls gradually, and quite regularly, from 2·25 to 2·02. Using the above values for *n* and *k*, the average deviation between the calculated and the observed figures is 0·77 per cent., while it is 3·44 per cent. (four and a-half times as great) if the area be calculated in per cent. of body weight. If the value of *n* be taken as 0·70, *k* becomes 6·98, and if *n* be 0·72, *k* is 6·93.

As regards the question of sex it is to be observed that, in the present series of observations, the average *k* for male animals is somewhat larger than average *k* for the females. That is to say, the *females* had slightly *wider* tracheas than the *males*.

In Table VII are tabulated the main results obtained, in such a manner as to show at a glance the range of weight, the best *n*, the value of *k*, the percentage deviation, and so forth, for each species of animal, both grouped and ungrouped. It will be seen from the averages brought out at the foot of the table that, taking the species together, the average percentage deviation for the individual animals between the calculated and the observed figures for the tracheal area is 9·02, when the calculation is made in terms of the body surface, while it is 14·23 when the area is expressed as a percentage of the body weight. The corresponding figures for the grouped animals are 2·5 and 12·28 respectively, a deviation nearly five times as large.

Just as was seen in our measurements of the aorta, the method used in measuring these tracheas is, in the nature of the case, much less exact than that employed by us in measuring the blood volume, and therefore gives much larger figures for the percentage deviation, yet this deviation is found to be reduced to very nearly the same extent in each case by grouping the animals; of course, reckoning by the body surface throughout. Thus, in the present instance, the ratio between individual and grouped percentage deviations is 9·02/2·5, *i.e.* 3·6, while in the other two cases it was shown to be 3·2.

The mean deviation between the calculated and the observed values, as determined by the method of least squares for the three species (grouped individuals), is 7·08 per cent. for the guinea-pigs, 1·58 per cent. for the rabbits, and 1·16 per cent. for the ptarmigan. The average of these figures is 3·27 per cent. The corresponding mean deviations when the tracheal area is calculated in percentage of body weight are for the guinea-pigs 33·42 per cent., for the rabbits 14·32 per cent., and for the ptarmigan 4·28 per cent., giving an average of 17·34 per cent.

Table VII.—Percentage Deviations, Constants, etc.

Animal.	Range of weight.	Weight of heaviest animal in terms of weight of lightest.	Best n.	k.			Average percentage deviation.				Deviation by weight divided by deviation by best n.
				$n = 0·70$.	$n = 0·71$.	$n = 0·72$.	$n = 0·70$.	$n = 0·71$.	$n = 0·72$.	As percentage of body weight.	
	grms.										
Guinea-pig :											
Individual	70–640	9·14	0·72	—	21·5	23·4	—	—	18·2	25·1	1·38
Grouped	70–630	9·0	0·71	20·1	21·3	22·5	—	5·46	5·76	22·49	4·12
Rabbit (tame) :											
Individual	940–2970	3·16	0·70	12·5	13·4	—	6·43	—	—	13·39	2·17
Grouped	955–2860	3·0	0·70	12·5	13·4	—	1·28	—	—	10·91	8·52
Ptarmigan :											
Individual	460–710	1·54	0·71	7·0	7·45	7·93	—	2·42	—	4·2	1·74
Grouped	465–670	1·44	0·71	6·98	7·44	7·93	—	0·77	—	3·44	4·47
Average percentage deviation taking best n { individuals								9·02		14·23	
grouped								2·5		12·28	

The ratio between these figures (3·27 and 17·34) is also almost exactly the same as that which was found between the corresponding figures in the case of the blood volume (2), although in that case the mean deviations themselves were much smaller (namely, 1·39 and 7·82 per cent. respectively), owing to the much greater intrinsic accuracy of the technique.

From these figures it is seen that if a series of observations of the tracheal area are made and averaged, it follows that if the difference between this average and the theoretical value given by our formula is as much as **7** per cent. the tracheal area is probably abnormal, and if it amounted to about **10** per cent. it would be almost certain that the trachea was abnormally large or small. But if the measurements were expressed in percentage of body weight it would only be possible to say with the same degree of certainty that the tracheal area of the animal was abnormal when it differed from the calculated value by **50** per cent. or more.

The inter-relation of the various constants (for surface, blood, aorta, and trachea) given in the present and in previous papers, together with its significance, will be dealt with in a later communication. But it may here be pointed out that the value of n in the expression $W^n/a = k$ has now been shown to be 0·70–0·72 for the *surface area* of *three* different species of mammals, for the *blood volume* of *six* mammals, for the *aortic area* of *four* species of mammals and *four* species of birds, and for the *tracheal area* of *two* mammals and *one* bird. Accordingly we regard our formula $W^n/a = k$ as a rational formula indicating that the blood volume, the aortic area, and the tracheal area are all proportional to the body surface in warm-blooded animals. From an examination of the large number of data which we have now collected it appears that if one desires rapidly to compare a series of individual observations by means of the formula $W^n/a = k$, the value of k may readily be determined approximately by using the power $\frac{2}{3}$ (= 0·67) or the power $\frac{3}{4}$ (= 0·75) instead of the accurate value of n (0·70–0·72). The results thus obtained will be approximately correct over a moderate range of weight. But as the range of weight increases the results deviate from the true values, and those obtained with $n = \frac{2}{3}$ deviate more rapidly than those obtained with $n = \frac{3}{4}$.

The difference in the relative accuracy of the results given by these two values of n has been ascertained in the following manner:—The percentage deviations for the surface, the blood volume, the aortic area, and the tracheal area for four different species of animal, with "best n," with $n = \frac{2}{3}$, and with $n = \frac{3}{4}$, were tabulated, and the figures for these three values of n averaged. The final average deviations thus obtained were 2·52 per cent. with n taken as 0·70–0·72, 3·25 with n taken as $\frac{3}{4}$, and 3·81 with n taken as $\frac{2}{3}$. Thus it appears that the value $\frac{3}{4}$ for n gives a deviation 1·29 times as great as that obtained with the best n, while if the value of n is taken as $\frac{2}{3}$ the deviation is 1·51 times as great as that with the best n.

Accordingly, it is clear that if an approximate value of n is employed for the sake of convenience in rapid calculation it is, as a rule, preferable to use the value $\frac{3}{4}$ rather than $\frac{2}{3}$. But wherever observations covering a wide range of weight are concerned it is essential to make use of the "best n" in order to obtain reliable results. In this connection it may be noted incidentally as a point of interest that the ratio between the deviations just quoted for $n = \frac{2}{3}$ and $n =$ best n, namely, 3·81/2·52 (*i.e.* 1·51), proves to be practically identical with the corresponding ratio already given elsewhere (2) in the case of the blood volume, namely, 2·08/1·39 (*i.e.* 1·5).

Conclusion.

Within a wide range of weight in any given species of warm-blooded animal the sectional area of the lumen of the trachea is proportional to the body surface, and can be calculated from the body weight by means of the formula $\mathrm{T} = \mathrm{W}^n/k$, where n has the value 0·70–0·72 and k is the constant to be ascertained for each particular species.

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Notes on the Life-History of Trypanosoma gambiense, *etc.*

By Muriel Robertson.

(Communicated by the Tropical Diseases Committee of the Royal Society.
Received September 28,—Read December 5, 1912.)

(Abstract.)

The following is a brief account of some of the more salient features in the life-cycle of *Trypanosoma gambiense.* The results are drawn from a large number of experiments carried out at the Mpumu Laboratory in 1911 and 1912. The present paper is in the nature of a very brief synopsis, and is not a full account of the experiments and conclusions.

I. *Endogenous Cycle in the Blood.*

The part of the life-history of *T. gambiense* spent in the vertebrate—the experiments were carried out with monkeys—is characterised, as is well known, by a marked fluctuation in the numbers of parasites present in the blood. The individuals show a wide range of variation in length and breadth. During the depressed periods, the few parasites present are of the short, relatively broad type. Periods of increase are characterised by the appearance in addition of the intermediate and long slender forms. The latter are the individuals about to divide. The short form may be looked upon as the adult blood-type, and is usually the most numerous form present, except during the periods of rapid multiplication. These periods set in regularly in every revolution of the cycle during the earlier months of the disease ; their recurrence is less marked in the later stages.

The short forms appear to be responsible for carrying on the infection in the *Glossina,* and the blood of a monkey is only infective to fly when these forms are present in sufficient numbers and in a suitable physiological condition, *i.e.* not suffering from exhaustion. Intracellular multiplicative phases do not occur in the lung, liver, or spleen of monkeys.

Rounded, non-flagellate individuals are occasionally found in the liver and lung, apparently between the cells, but it is not perfectly clear whether they may not be in rare cases within the cells. They appear at the time when the trypanosomes are being destroyed, before the depressed periods of the endogenous cycle, but have only been found in a teeming infection examined during the earlier months of the disease. These creatures are apparently about to be destroyed, but their survival in very small numbers as latent forms cannot be entirely excluded.

No sexual differentiation of any kind is to be observed among the blood-types. The differentiation into long and short forms is a phenomenon of growth and division, and is not an expression of sex.

A wet fixation of the blood-films in corrosive alcohol, and subsequent staining and mounting without drying in air, gives the nuclear picture shown in figs. 25 and 26. This is in accord with the observations upon live specimens, and is identical with that found in other trypanosomes studied by similar methods. A division-stage is shown in fig. 27.

II. *Exogenous Cycle in the Fly.*

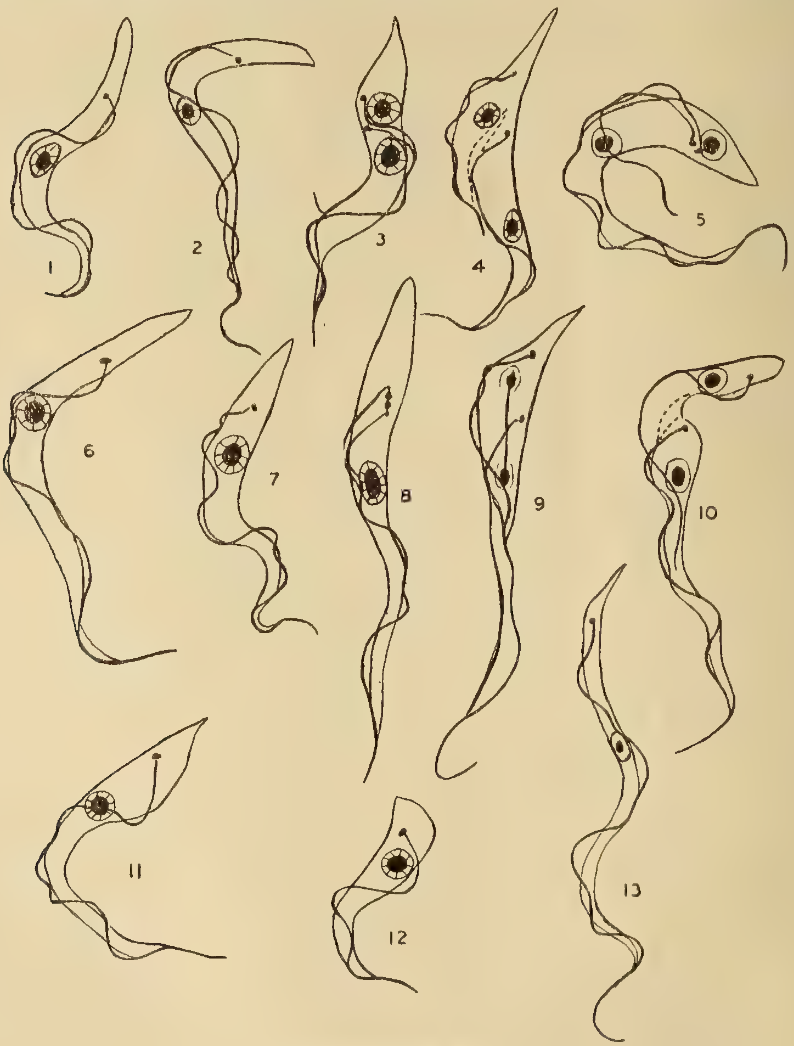
A great deal of information can be obtained from a close study of the conditions in the early days of the cycle as to what are the factors inhibiting the development of the trypanosomes in the *Glossina*. I do not, however, propose to discuss these here; suffice it for my present purpose to take those cases in which trypanosomes have established themselves, and to trace the usual development.

While the series of changes undergone in the *Glossina* up to the time when it becomes infective to clean vertebrates is very definite and constant, nevertheless, the duration in time of this cycle varies in different cases within the limits of more than a fortnight. This must naturally be borne in mind when considering the successive stages in the cycle of any given fly.

The trypanosomes never attach themselves while in the gut, nor do they ever disappear from this situation at any period; the development occurs free in the lumen of the alimentary canal from the very start. At no period do the parasites enter the body-cells of the host, nor do they penetrate through the gut-wall into the body cavity.

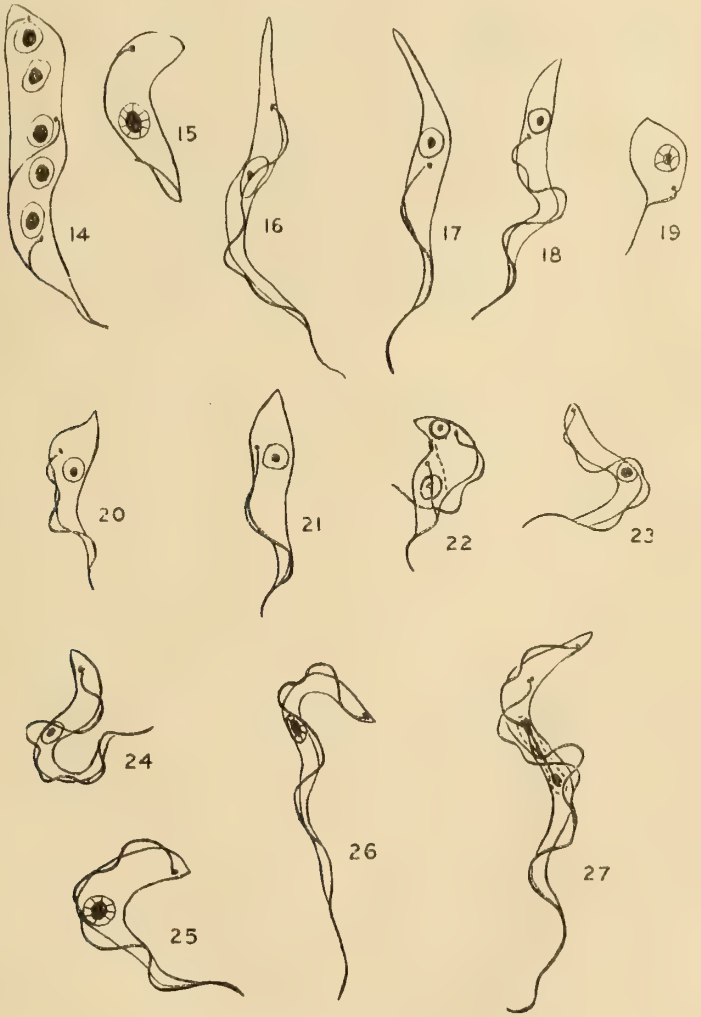
The earliest processes that take place in the fly are characterised by a slight and rather indefinite change of form (figs. 1 and 2). Broad, slender and degenerating specimens are all present, but only the broader types are ever found in division at this early stage. These first divisions (figs. 3 and 5) are remarkable in that they show a suppressed crithidial phase in the young individual. This disappears before the separation of the two products (fig. 4). The peculiarity just noticed does not occur in the later divisions, and has never been observed after the 10th day. The gut stages do not show any other crithidial phase. The trypanosomes usually start developing in the middle or posterior intestine (mid-gut), and by the 7th to 10th day there are a large number of trypanosomes present, showing the general features depicted in figs. 6–12. Division goes on rapidly, and the nature of this process is shown in figs. 8–10.

It will be observed than the granule at the base of the flagellum (blepharoplast of Minchin) plays the *rôle* of centrosome in the division of the kinetonucleus. It must also be noted that the division is not really longitudinal but practically transverse, the plane of division being at right angles to the long axis of the parent individual (fig. 10). Division is often unequal.



Magnification 2500.

Multiplication proceeds until the whole of the middle and hinder and part of the anterior intestine is filled with parasites. Very slender long forms are developed about this period (8th to 18th day or thereabouts) and these gradually pass forward into the proventriculus. This slender type (fig. 13) is essentially the proventricular form and is the culmination of the development in the gut. The trypanosomes may overflow into the sucking-stomach or crop, but are not permanently established there. The flagellates are, moreover, unable to retain their position in the proventriculus if the fly is subjected to a fast of a considerable duration, such as any period exceeding two or three days.



Magnification 2500.

Multiple Forms.—Up to about the 10th or 15th day of the cycle multiple forms such as those shown in fig. 14 may be seen in certain cases. The evidence is largely in favour of these being degenerative stages but is not sufficient to entirely exclude the possibility that some of them (compare fig. 15) may not be involution-forms or resting phases capable of further development and activity. Proventricular forms when injected into clean monkeys do not produce infection.

Invasion of the Salivary Glands.—The long slender forms from the proventriculus come forward into the hypopharynx in small numbers at a time and may be found lying free in this situation in carefully dissected specimens before the glands are infected. From the hypopharynx they pass back along the narrow ducts of the salivary glands and it is not at all a rare occurrence to find trypanosomes in the ducts of the glands in 16- to 30-day flies when the rest of the glands show no flagellates at all. The trypanosomes reach the glands as long slender forms and attach themselves where the duct joins on to the slightly broader part which leads to the glandular portion proper. They become much shortened and very much broader and assume the crithidial condition shown in figs. 16–21. They break free occasionally but seem to attach themselves again. Multiplication (fig. 22) occurs and the trypanosomes gradually invade the whole gland; new specimens keep on arriving from the hypopharynx. The short dumpy crithidial forms develop into trypanosomes almost identical with the blood-forms but often a little below the normal adult length (figs. 23 and 24). These trypanosomes are found swimming free in the lumen of the glands and there is the strongest presumptive evidence for considering that these are the types that produce the infection in the vertebrate.

Not only is this second development in the glands necessary to produce an infective fly, but from a number of considerations, amongst others the appearance here of the very clear and definite crithidial stages, it may be held that the development in the glands is the really essential part of the whole cycle. The development in the gut may be considered as a somewhat indifferent multiplication—a mechanical device to enable the trypanosomes to establish themselves in sufficient numbers in contact with the salivary fluid, which alone, in the *Glossina*, seems able to stimulate the trypanosomes to the apparently essential reversion to the crithidial type.

Conjugation.—Sexual differentiation has not been observed at any part of the cycle; this is not, however, a characteristic feature of flagellate life-histories. Isogamy seems to be usual among the group. The direct evidence of conjugation is slight and not sufficiently convincing. General theoretical considerations are, however, very strongly in favour of some such process occurring, and from comparative evidence drawn from the consideration of the cycles of *T. nanum* and *T. vivax* it seems possible that the sexual part of the cycle might take place in the salivary glands.

It is obvious that much of the foregoing work has been simply to carry somewhat further the researches of Minchin, Roubaud, Bruce, and Kleine, more especially those of the two last-named workers. There are no serious discrepancies between the cycle in the fly sketched by Bruce, Hamerton, and Bateman and that described above, except that I consider the fly history to be in reality a double development. In many points my work is also in agreement with that of Kleine and Taute,* except that I do not consider that the "male" forms described by them play any important part in the cycle. A further discrepancy consists in the view held by the latter authors at the time of writing their paper in regard to the salivary gland phases being a non-essential part of the cycle. My interpretation of the endogenous cycle in the blood of the vertebrate is at present, so far as I am aware, unconfirmed by other workers, largely, I imagine, owing to the fact that the interest has been concentrated for some time past on the appearances in the fly rather than on those in the vertebrate.

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* 'Arbeiten aus dem Kaiserlichen Gesundheitsamte,' vol. 31, part 2.

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On the Comparative Anatomy and Affinities of the Araucarineæ.

By Prof. Robert Boyd Thomson, University of Toronto.

(Communicated by Dr. D. H. Scott, F.R.S. Received September 14,—
Read November 14, 1912.)

(Abstract.)

From a study of the anatomy of the different regions of the plant, evidence is found of the relationship of the Araucarineæ to the Cordaitales.

In the first place, the presence of a leaf gap opposite the outgoing foliar trace, in all forms whether the leaf be large or small, is taken as indicating the Pteropsid ancestry of these forms and is considered of sufficient importance to preclude the possibility of the Lycopsid connection of the Araucarineæ, of which view Seward has been the recent exponent. The presence of a gap in the cone and in the seedling seems to put the question beyond doubt, since this indicates the *ancestral presence* of a leaf gap.

One evidence of relationship to the Cordaitales is found in the retention of Cordaitean pitting of the tracheids in the different regions of the plant which are recognised as primitive, in the cone especially, where the pitting may be as much as 5-seriate, the pits, alternate, hexagonal and extending from end to end of the tracheid as in the Pteridosperms and the primitive members of the

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* 'Arbeiten aus dem Kaiserlichen Gesundheitsamte,' vol. 31, part 2.

Cordaitales. In medullary ray structure, too, all the forms are Cordaitean—the ray cells thin walled and with numerous pits on the tracheids where the cells come into contact with them. In both groups the rays are resinous but devoid of resin canals. In both there are ligneous parenchyma cells and resinous tracheids in the secondary wood. The latter are considered the ancestral form of the resin tissue from which the other types in the secondary wood of the conifer series have been derived. With regard to leaf traces, too, both groups agree—the trace may be single or double even while it is still in the secondary wood. In the leaf there is centripetal primary wood directly opposite the protoxylem.

In no case was there found in the primitive regions of the Araucarian forms any indication of Abietinean structure, which would be expected if the view of the Abietinean ancestry of Araucarineæ which is advocated by Jeffrey be correct. In contrast to this, in the primitive regions of the Abietineæ there are evidences of Araucarian pitting, etc. In addition, evidence is advanced to show that the transitional forms upon which the claim for the greater age of the Abietineæ is based, indicate rather the derivation of the Abietineæ from the Araucarineæ or Cordaitales. Of special interest in this connection is the evidence that the traumatic resin canals of Araucariopitys are of a primitive type and in the process of *acquirement*. The determining points are the resemblance of these to both the normal ones of the cone and to the traumatic series of the vegetative parts of the living pine and their difference from those of such a form as Abies, where it has been shown that resin canals are *revived* by injury. The Abietinean theory of the ancestry of the Araucarineæ recognises only traumatic series of the "revival" type, and yet there is no record of authentic Abietinean forms, as has been recently shown, either in or previous to the Triassic, in which the first Araucarian supposed to be derived from the Abietineæ (Woodworthia) makes its appearance. Thus geologically as well as structurally the superior antiquity of the Araucarineæ rests upon a very firm basis. This basis is made the more secure by the practically unbroken sequence of forms with essentially Araucarian structure right up to the Triassic.

In every respect confirmation of the old view has been found, which regards the Araucarineæ as anatomically very closely associated with the Cordaitales.

The Relation of the Islets of Langerhans to the Pancreatic Acini under Various Conditions of Secretory Activity.

By John Homans, M.D. Boston.

(Communicated by Prof. E. H. Starling, F.R.S. Received October 1, 1912,—Read January 23, 1913.)

(From the Institute of Physiology, University College, London.)

[Plates 2 and 3.]

The object of this research* was to examine the relations of the islets of Langerhans to the pancreatic acini under the following conditions: first, at a time when the pancreatic tissue had been cut down to a very small amount, and second, when the gland had been exhausted by the action of secretin.

Under the first of these conditions it was expected that, as the removal of the entire pancreas was inconsistent with life, the removal of all but a little might lead to the hypertrophy of one or both elements in the part remaining, and throw some light on the possibility of a change of acinous to islet tissue or the reverse. As a control to this procedure the pancreatic ducts were ligatured in one animal because the pancreatic acini rather than the islets are generally supposed to degenerate under these circumstances. In the second series of experiments—exhaustion of the gland by secretin—it was intended to test the assumption by Dale, Vincent and Thompson, and others, that this proceeding induces a change of acinous into islet tissue.

It is hardly necessary for me to summarise the literature of this subject beyond repeating the most typical of the opposing views as to the functional and anatomical independence of the islets and their relation to acinous tissue. Ranged on the side that the islets are more or less interchangeable with acini are a number of investigators, whose views, however, show important differences of detail. Lewaschew (1886) holds that as a result of exhaustion acinous cells become converted into islet tissue and are capable of re-forming after rest. Laguesse (1893–1911) maintains a somewhat similar view of "balancement" between the tissues, both on embryologic grounds and as a result of a long series of anatomical and experimental observations. He considers, however, that the islets have an independent function. Mankowski (1902) considers that the islet is the highest stage of acinous cell activity into which all such cells must go. Dale (1904) sees, as a result of exhaustion,

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* The research was carried out with the aid of funds from the Peter Bent Brigham Hospital of Boston, U.S.A.

starvation, and duct ligation, the formation of new islets from acini. Karakaschiff (1904–1906), in a study of diabetes in man, finds that islets are without function except to form acini. Herxheimer (1906), in a similar study, concludes that islets are made from acini as a regenerative process. Vincent and Thompson (1907) agree with Dale. Fischer (1912) believes that the islet cells are able rapidly to change in function and appearance.

From the point of view of independence of function, the opinions of those who, following the lead of Mering and Minkowski, have endeavoured to discover which part of the pancreas is concerned with the sugar function are more in agreement. Thus Schultze (1900), Ssobolew (1902), Sauerbeck (1904), Tschassownikow (1905), Tiberti (1909), and Laguesse (1911) substantially agree that duct ligation does not produce diabetes. Of these, all but Sauerbeck find that islet tissue is not destroyed in the process, and this conclusion, taken into consideration with the occasional finding by Opie (1901) and others of extensive islet disease in human diabetes, furnishes a strong argument in favour of the functional independence of this tissue. Partial extirpations and pancreatic grafts by Laguesse (1902), Kyrle (1908), Tiberti and Franchetti (1909) have led to similar conclusions. It must be admitted, however, that except for Ssobolew (1902) no one has been able to see as a result of stimulation with carbohydrates or phloridzin any changes in the islet cell indicating stages of functional activity [Tiberti (1909), Frugini and Stradiotti (1909)].

The supporters of the view that islets are unchangeable anatomic structures bring evidence from a number of sources. Embryologic investigations by Pearce (1903), Küster (1904), Helly (1905), and Weichselbaum and Kyrle (1909) point to the origin of islets in early fœtal life from primitive duct tissue and to their subsequent anatomic independence. None of these investigators have been able to confirm the observations of Laguesse (1895–6) in regard to the disappearance of his "primary" islets and the substitution of "secondary" islets from acinous tissue.

Other investigators, by methods similar to those employed by Lewaschew, Minkowski and Dale, have come to conclusions diametrically opposite to theirs, a fact which suggests that the matter is, to a great extent, one of interpretation of the histologic appearances and of microscopic technique. Thus Diamare (1899) finds that the islets contain two kinds of specific granules, and have an important sugar function. Opie (1900) finds no increase in the number of islets after prolonged stimulation with pilocarpin. Ssobolew (1902) holds the islets to be independent structurally as well as functionally. He sees specific granules in the islet cell. Dewitt (1906) finds a great variation in the normal number of islets, but no increase or change resulting from starvation, feeding, and so on. Lane (1907) shows that there are two kinds of granules in the islet cell, differing chemically from each other and from the zymogen of the acini.

Finally, Bensley (1911), in a most exhaustive study of the pancreas of the guinea-pig, has cleared up much of the doubt surrounding this subject. By the use of vital stains he shows that the islets can be readily identified and counted, and that their number is not influenced by starvation, or any known method of stimulation. He demonstrates, by the use of refined methods of fixation and by granule stains, that the islet cells have a structure distinct from that of the acinous cells, as well as from the duct and centroacinous cells with which they are likely to be confused, and among which they are frequently found. He points out with justice the error in the common practice of identifying the islets by purely negative means, that is, by fixations and stains which do not bring out their distinguishing characters. I have repeated some of his experiments with vital stains to satisfy myself of the fairness of his basis of identification, and this research would have little justification if it did not take a direction in which Bensley's work has not carried him. I have borrowed freely from his methods.

Technique.

The animals used in this research were dogs and guinea-pigs. No especial selection of animals was made. The experiments* include two exhaustions of the dog's pancreas by secretin, five nearly complete extirpations and one duct ligation in the dog, and three pancreas exhaustions in the guinea-pig by purified secretin. In addition, a number of injections of neutral red, methylene blue, and pyronin have been made in guinea-pigs, according to the methods of Bensley.

Fixing and Staining Methods.—All specimens were removed immediately after the animals had been killed by bleeding under anæsthetics. Pieces of pancreas not more than 2 mm. in thickness were placed at once in a number of fixative solutions and stained by various methods.

I. Acetic-osmic-bichromate (Bensley).

Osmic acid	4 c.c.
Potassium bichromate	15 c.c.
Acetic acid	2 drops.

Tissues fixed from 16 to 24 hours were washed in water and passed through alcohol to paraffin. Ordinary tissues were cleared in benzol before paraffin, but pieces containing considerable scar tissue were passed instead through carbon bi-sulphide for greater ease of sectioning. Sections cut less than 4 micra in thickness were stained by the acid-fuchsin-methyl-green method advocated and fully described by Bensley.

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* All experiments in which the animals have been allowed to recover from the anæsthetic—five extirpations of the pancreas and one duct ligation in the dog—were performed by Prof. E. H. Starling. The exhaustion experiments were performed by the writer.

II. Aqueous-chrome-sublimate (Lane's method for B cells).

Potassium bichromate	2·5 grm.
Mercuric chloride	5 ,,
Distilled water	100 c.c.

Very small pieces of tissue were fixed for 12–24 hours, washed in water and passed through alcohol (including an iodine solution) to paraffin. These sections were stained for 24 hours in a 20-per-cent. solution of neutral gentian. (For the details of preparing this solution see Lane's or Bensley's article.)

III. Alcohol-chrome-sublimate (Lane's method for A cells).

Saturated alcoholic solution of mercuric chloride.
Potassium bichromate, 2·5 per cent.
(Equal parts.)

Tissues fixed in this solution for three to four hours with one change were washed in 50-per-cent. alcohol and passed rapidly through alcohols to paraffin. Neutral gentian stain.

(As this method produces considerable shrinkage, and as in my hands it has proved quite uncertain, especially in the dog, I have made little use of it.)

IV. Zenker's fluid.

Tissues treated were stained with methylene blue and eosin or a similar combination. This technique does away with the granules in the acini and islets as well, and may be used to show the " negative " of the granule stains.

Demonstration of Islets by Vital Staining Methods.—I have repeated a number of Bensley's experiments to satisfy myself that they are valuable in less skilled hands than his. Only the neutral red, pyronin and methylene blue methods have been used. A full description will be found in Bensley's article.

Operative Procedure.

1. *Partial Removal of the Pancreas.*—Under morphine and ether the pancreas, with the exception of about one-tenth at the duodenal end, was removed by blunt dissection (Hédon's method) without ligation of blood vessels. There was no bleeding. The cut end of the remaining part, generally about 2 cm. in length, was tied off with silk and was either transplanted with its blood supply outside the oblique muscles through a slit in the muscle or left *in situ.*

2. *Ligation of the Ducts.*—Under morphine and ether the large duct was isolated for ½ cm. and divided between two silk ligatures. The pancreas in this region was separated from the duodenum, and omentum was inserted between the gland and the bowel. To divide the smaller duct the tissues about the common bile duct were ligated on both sides and divided. The gland was further separated from the bowel and omentum inserted between.

3. *Exhaustion of the Dog's Pancreas by Secretin.*—The animals were fed as usual the afternoon before the experiment. They were anæsthetised by morphine and ether. A secretin solution prepared from small intestines by the method of Bayliss and Starling was allowed to run into the external jugular vein by means of a cannula. A cannula was tied into the pancreatic duct and the juice measured. A piece of pancreas was isolated at the beginning of the experiment as a control of the exhaustion. The stimulation was kept up until the pancreas failed to respond to the stimulation or until the dog died.

4. *Exhaustion of the Guinea-Pig's Pancreas by Purified Secretin.*—In order to do away with the fatal depressing effect of secretin on the guinea-pig, purified secretin prepared by the method of Dale and Laidlaw* or Stepp was used. The animal was anæsthetised by morphine and ether. The method of introducing the secretin was the same as in the dog, but no cannula was tied into the pancreatic duct. The degree of exhaustion was estimated by the examination of a piece of pancreas under the microscope.

The Normal Resting Pancreas of the Dog.

A detailed description of the pancreas is superfluous, but in connection with the accompanying illustration (fig. 1, Plate 2) a brief description of the different kind of cells is necessary as a base with which the experimental conditions produced may be compared. The anilin-acid-fuchsin-methyl-green stain after osmic-chrome-acetic fixation has proved, in my hands, so much more successful than any other for the dog's pancreas that it is upon this that most of my conclusions are based.

By this technique the normal charged acinous cell shows the characteristic zymogen granules stained bright red, the mitochondrial filaments the same colour, while the protoplasm of the cell takes a smooth greenish tint. In association with many acini, the centroacinous cells can be distinguished by their clear uncoloured protoplasm and their oddly shaped red stained granular elements. Some of these cells are indicated in the illustrations, and it is easily seen that they are, apart from the shape of their granules, strikingly like the clear granular cells of the islet. The third cell which it is important to recognise is that of the ducts, and especially the finer ducts, or duct-like rows of cells, which are so often found associated with the islets. These cells are usually oval in shape and often considerably compressed. Their nucleus is central and their clear protoplasm dotted with a variable quantity of mitochondrial elements. They have, like centroacinous cells, a distinct resemblance to islet cells and, as Bensley has pointed out, the latter are often found among them.

The islet cells may be easily divided into the two varieties which have been noted by many investigators, and to which Lane has given the name A and B cells accordingly as their granules are fixed respectively by alcoholic or watery solutions. With the acid-fuchsin-methyl-green stain the B cells, which form the vast majority, take a slate blue colour. They are usually smaller than the A cells, which show red granules and a clear uncoloured protoplasm. Both cells possess mitochondrial elements. In the B cells which have not taken up sufficient of the methyl green to give them their characteristic blue colour, the mitochondrial filaments give the impression of red granules, but in the heavily stained cells they hardly show.

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* I am much indebted to Dr. Dale for a supply of secretin prepared in this way.

The nuclei of the various cells present less characteristic differences than do the granules. Those of the acinous cells do not always show a bright red nucleolus. On the other hand, the chromatin which they contain is often arranged like that in the islet nuclei. Moreover, the islet nuclei may often contain what appears to be a typical nucleolus. Accordingly, for purposes of differentiation, I have given little attention to the nuclei of the different cells.

I shall describe the appearance of islet tissue stained with neutral gentian in connection with pancreas exhaustion experiments in the guinea-pig. In the dog, neutral gentian, though it stains beautifully the zymogen granules, has proved rather unsatisfactory for the islet granules. In the guinea-pig, on the other hand, it gives the most striking pictures.

The Exhausted Pancreas of the Dog.

Of the two exhaustion experiments performed on the dog only one was completely successful. In this the flow of pancreatic juice was very free, about 210 c.c. being secreted in the course of nine hours. At the end of this time, though the flow had not ceased, the pancreas appeared rosy and translucent in contrast to the opaque appearance of the small segment tied off for comparison.

Examination of the discharged organ (fig. 2, Plate 2) shows the exhaustion to be fairly complete. There is no evidence of any transition of acinous to islet tissue. The acinous cells have for the most part lost their zymogen granules. In consequence, the mitochondrial filaments appear more prominent than usual, but the protoplasm of the cells, though somewhat vacuolated, still takes the characteristic smooth green stain.

The islet shown in the figure is well charged with granules. Whether the gathering of these granules along the capillaries is an effect of secretin stimulation I do not know, but as most of the islets do not present this appearance, and as it has been noted in the resting gland, I believe that it is accidental. The most noteworthy change in the relation of the islet to the surrounding acinous tissue is that the exhaustion of the zymogen in the surrounding pancreas causes the islet to appear more sharply marked off than usual. This is in strong contrast to preparations of the same organ fixed in Zenker's fluid, and stained with eosin and toluidine blue, when the islets, being merely negatively stained, can be distinguished with some difficulty from the more exhausted portions of the acinous tissue.

The Exhausted Pancreas of the Guinea-Pig.

Three exhaustion experiments were performed on guinea-pigs, in only one of which could the animal be kept alive long enough to produce any obvious exhaustion of the gland. This animal lived for six hours under stimulation by secretin purified by the method of Stepp. The appearance of the gland treated by the acid-fuchsin-methyl-green method differs in no essential way from that of the dog, but under the neutral gentian stain the islets are marked off with a distinctness which I have not been able to obtain with a similar stain of the dog's pancreas.

Figs. 3 and 4 show an islet with the surrounding tissue in a charged gland and after exhaustion. By this method the zymogen granules of the acinous cells are stained a deep lilac and the protoplasm a pale purple. No mitochondrial elements are seen, and the centroacinous and duct cells likewise fail to stain.

In the islet of the charged gland the B cells are stained blue, the fine uniform granules alone taking the colour. No mitochondrial elements are to be seen. It therefore appears probable that the red rods and granules which appear among the slate blue granules of the B cells of the acid-fuchsin preparations are in reality mitochondrial in nature and not granular. The A cells, or those which stain red with acid-fuchsin, fail here to take a positive stain. They appear as faint yellowish to purple homogeneous masses with an indistinguishable outline.

In the exhausted gland the zymogen granules are greatly reduced. Occasional small masses are present, especially close to the surface of a lobule, but about the islet shown in fig. 4 (Plate 3) they have almost completely disappeared. The protoplasm of the acinous cell, however, takes the same stain as in the charged organ, though it is often considerably vacuolated. Among the acini a cell appears to which Bensley has called attention. Several of these are shown in fig. 4 about the border of the islet, though they are no less common in other parts of the lobule. This cell is filled with perfectly round, uniform, purple granules, considerably smaller than zymogen and distinctly larger than those of the islet. I have found cells similar to these in specimens of discharged gland stained by acid-fuchsin. Here the granular stain is uniformly red, but in addition the cell protoplasm also takes a diffuse red stain. Such cells have a typical acinous arrangement, and rarely one is found with some of the diffuse green colour of the normal cell. I have not been able to find such cells in the acid-fuchsin preparations of resting glands, but have found one or two in neutral gentian stains of similar material. They resemble those which Bensley describes as a result of a *post-mortem* change peculiar to the guinea-pig, and which he holds to be the same cells described as a transition form by Mankowski. At first I was inclined to disagree with Bensley, and to consider the cell a result of exhaustion rather than of a *post-mortem* process, for while I find them abundant in exhausted glands, I have rarely seen one in a normal organ. It seems to me, however, that as the circulation in animals subjected to secretin stimulation becomes in the last half-hour very poor, and as their temperature becomes considerably subnormal, the change may be partly *ante-mortem* degeneration. Bensley's evidence that such cells can be produced as a result of *post-mortem* changes is very complete. That they are produced by exhaustion is suggested by my preparations, but not proven. Moreover, they have not been noted in the dog. In any case they furnish the only suggestion of a possible transition of acinous to islet cells which I have been able to find in the exhausted pancreas of the guinea-pig.

Demonstration of Islets and Ducts by Vital Stains.

I have made, perhaps, half-a-dozen injections of neutral red, pyronin, and methylene blue, according to Bensley's method, and I can only say here that the results fully bear out Bensley's contention that the islets have a distinct staining reaction of their own. In the best preparations, the individual cells can be distinguished, and, when the neutral red injection is combined with pyronin, the relation of the islets to the ducts is quite obvious. Though I have only made one attempt to demonstrate the islets in the exhausted gland, the clearness of outline, size, and number of the islets was quite the same as in the resting gland. For a full account of this technique and its results in exhaustion and starvation, the reader is referred to Bensley's work.

Nearly Complete Removal of the Pancreas in the Dog.

The choice of the duodenal end of the pancreas as the part to be studied was made with the full knowledge that the number of islets left would be comparatively small. It was thought, however, that if an amount of this pancreatic tissue could be left, such as would barely support life, a better idea of the relation between acinous and islet tissue could be gained than by the use of the splenic end, in which the islets are known to be much more abundant. Although these experiments were planned primarily for this purpose, the importance of the relation of the histological changes to the carbohydrate metabolism made it necessary to observe the general condition of the animals, and especially the amount of sugar appearing in the urine. Accordingly, a brief protocol of each experiment is given, with a summary of the histological findings at its termination. The observations are arranged according to the number of days the animals were allowed to survive operation.

Experiment 1.—Young female. Weight, 5·1 kgrm. Rather fat. Morphine, ether. Removal of the pancreas except for a segment at the duodenal end, measuring 1·5 × 1·5 cm., which is tied off from the intestine and the rest of the organ with silk. With its blood supply intact it is transplanted through a slit in the oblique muscles to a space outside the muscles. Good recovery from operation, but the animal, though it does not look ill, remains quiet for the next three days. Urine for the second 24 hours following operation 250 c.c. It contains 3 per cent. sugar. On the morning of the fourth day the animal looks ill, has lost fat, and a fluctuating mass is felt at the site of the graft. Killed by bleeding under ether. Examination shows a thin, yellowish fluid surrounding the graft, which appears white and opaque. No pancreatic remains found in the abdomen.

Microscopic Examination.—Superficial infection of the graft. Acinous cells highly charged with zymogen. Apparent slight increase of duct tissue. The islets appear normal in size and shape. The B cells are for the most part small, and very few of the typical slate blue granules are seen. The A cells stand out darkly stained and prominent.

Experiment 2.—Adult female. Weight, 8 kgrm. Good condition. Morphine, ether. Removal of the pancreas except for a piece 2 × 2 cm. tied off with silk at the duodenal end. Transplanted with its blood supply outside the oblique muscles. Considerable handling of the transplant. Good recovery from ether. Twelve hours' specimen of urine on night of third day contained 3·35 per cent. of sugar.

7th day.—Animal thin but seems lively and eats and drinks well. Urine for 17 hours (550 c.c.) contains 3·95 per cent. of sugar.

8th day.—Wound puffy and graft swollen.

10th day.—Site of graft looks better. Animal lively. Sugar, 1·9 per cent. (single specimen).

13th day.—24 hours' urine contains 4·5 per cent. of sugar. The graft looks normal, and the animal seems lively and well.

16th day.—Killed by bleeding under ether.

Examination shows that the site of the graft is clean. A moderate amount of scar tissue surrounds the graft, which appears about the same extent as at operation, but considerably thicker; white, opaque, and very firm on section. No pancreatic remains found in abdomen.

Microscopic Examination.—The acinous cells are highly charged with zymogen. They appear slightly shrunken, though there is no evident destruction. There is a great relative, and probably absolute, increase of small ducts and centroacinous cells. No mitotic figures are found. The islets appear considerably altered. The B cells stain poorly, showing few slate blue granules. They contain some red and some uncoloured granular material, and are generally shrunken.

Experiment 3.—Adult female. Weight, 9·6 kgrm. Good condition. Morphine, ether. Removal of the pancreas except for a piece 2 × 2 cm. tied off with silk at duodenal end and left in place with blood supply intact. Slow recovery from ether, and animal seems very quiet on the day following.

3rd day.—Animal lively and well.

7th day.—Animal eats and drinks well. Urine contains no sugar.

10th day.—Weight, 9 kgrm. Animal seems well.

20th day.—Weight, 8·75 kgrm. Animal seems well. Overnight urine contains 2 per cent. sugar (estimated).

26th day.—Animal in good condition. Killed by bleeding under ether.

Examination.—There is a little clear fluid about one end of the graft, which is thick, white, and opaque. The other end, or about one-half of the original fragment, is thin, grey, and scar-like, but shows the normal markings. No other pancreatic remains found in the abdomen.

Microscopic Examination.—Sections from the swollen opaque end differ in no essential particular from those of Experiment 2 (16-day graft) except that the islets are rather better preserved. The B cells are very few in number, which takes away from the islet its characteristic appearance (fig. 6), but it is easy to identify. Sections from the atrophied end present appearances shown in fig. 5. The acinous cells still contain zymogen granules and stain characteristically, but they are shrunken and gathered into small compressed groups. There is an obvious relative increase of duct and centroacinous tissue. A few islets can be found, but they take almost none of the bluish stain characteristic of B cells, and as they thus resemble duct cells (compare figs. 5 and 6) they are not easy to identify. They are not, however, increased in size, nor is there any evidence of a change of acini into islet tissue. My impression is that the islets are undergoing destruction by the scar tissue.

EXPERIMENT 4.—Adult female. Weight, 6 kgrm. Rather thin. Morphine, ether. Duodenal end of gland tied off as usual and rest of pancreas removed. Transplantation of fragment 2 × 2 cm. with its blood supply intact outside the oblique muscles. Good recovery from ether. Although it has lost some weight the animal remains lively and well until just before it is killed.

8th day.—Urine contains no sugar. Graft plainly felt through skin, which moves freely over it.

29th day.—Animal seems well. Overnight urine (250 c.c.) contains 2·7 per cent. sugar.

33rd day.—The animal appears ill and has not eaten during the last 24 hours. Killed by bleeding under ether.

Examination.—Peritonitis following perforation from fæcal impaction (undigested meat). The graft is shrunken, grey, and scar-like, perhaps two-thirds its original size. A firm elastic cyst is embedded in it. The lobulation is still evident.

Microscopic Examination.—The whole graft is invaded by scar tissue. The acini are broken up into small groups. The acinous cells are generally well enough preserved to be identified, and contain many zymogen granules. On the whole the general colour of the protoplasm is less evident than usual, and only the group arrangement and the presence of typical zymogen granules allow the acinous cells to be distinguished. The duct and centroacinous cells seem to be increased in number, and when found in groups resemble islets. A great many cells scattered through the specimen have the appearance of islet cells, but on looking through 30–40 sections I have found no complete typical islets.

Duct Ligation.

EXPERIMENT 5.—Adult female. Weight, 7·3 kgrm. In good condition. Morphine, ether. Ligation of ducts. Good recovery from ether.

4th day.—24 hours' specimen of urine contains no sugar.

11th day.—Weight, 6·25 kgrm.

18th day.—Weight, 7 kgrm.

28th day.—Weight, 7·25 kgrm.

35th day.—No sugar in overnight urine. Weight, 7·75 kgrm. Animal seems fat and well. Killed by bleeding under ether.

Examination.—The pancreas is shrunken to two-thirds its original length. The whole splenic end is much narrowed and a little thickened. It appears pale and scar-like. The duodenal end is much the same. The region of the duct outlets for about 3 cm. is broad, thick, white, and opaque, resembling exactly the 16-day (Experiment 2) and part of the 26-day (Experiment 3) grafts. There is a little clear fluid in the tissue about this region, but no evidence of the re-establishment of the ducts or the escape of pancreatic juice into the bowel can be made out. (Probably some slight connection has been re-established, as the animal's digestion after the first two weeks became so much improved.)

Microscopic Examination.—The thick, opaque portion resembles that of Experiments 2 and 3 referred to above. The shrunken splenic end resembles in some degree the contracted portion of Experiment 3 and the whole of Experiment 4 (26- and 33-day grafts), but there is less increase of duct tissue and considerably less destruction of the acini. A number of large islets are present. These appear normal in every respect, and though their large size and number might lead to the suggestion that new islets had been formed, they are no more in evidence than would be expected in the splenic end after the acinous tissue had shrunk to perhaps one-half its usual volume.

Results of Nearly Complete Pancreas Removal.

These results are to be considered from two points of view: first, and most important, according to the altered appearance of the acini and islets, and second, according to the apparent relation of these appearances to the sugar function of the pancreas.

It is quite evident from my specimens that, in general, the principal changes which have occurred in the pancreatic remains are the increase of duct and centroacinous tissue, which appears to reach its height in the course of the first few weeks, and an invasion by scar tissue of the acini, and probably of the islets as well. Instead of an interchange of acinous and islet tissue, there is much more a suggestion of a relapsing of islet into duct tissue, and possibly a change of duct to islet. Even in the small fragments which have remained in the animals for several weeks, the acinous cells, though shrunken and considerably altered in their staining reaction, are comparatively easy to recognise. One distinguishes between acinous and islet tissue much more easily than between duct and islet tissue, a fact which might well be expected from a consideration of the embryology of the islets and their constant relation (Laguesse, Bensley) to the ducts in adult life.

There are present, then, in these specimens, a number of cells which might conceivably be either duct or islet, their grouping suggesting now one and now the other. In all the grafts, the islet cells, that is, the prevailing cells containing the fine slate-blue granules demonstrated by the acid-fuschin technique, the B cells of Lane and Bensley, tend to lose these granules while retaining their mitochondrial filaments, and so resemble strongly the cells of the finer ducts. In the earlier, less cicatrised grafts, such islets are easily distinguished, and often show only one or two normally stained B cells. In the more scar-like specimens, when the islet cells retain their grouping, and are associated with one or two slate blue cells, they are again easily distinguished, but, when no such granule cells are present, and especially when the islets are disintegrated, a positive identification of any one islet cell, as opposed to a duct cell, seems to me impossible. This alteration is illustrated in figs. 5 and 6. Fig. 6 shows an islet of the 26th day fragment (Experiment 3). Several of its cells are obviously B cells, well stained, but the greater part of the islet is not to be distinguished from the masses of duct cells shown in fig. 5, which is drawn from the same specimen.

I have been unable to determine whether this peculiarity is a result of degeneration or of over-activity, though I am inclined to attribute it to the latter. For, inasmuch as a very small amount of pancreatic tissue, and that portion containing the fewest islets, is left after operation, the islets, whatever their function, must be working at their maximum capacity. That their characteristic specific granules should therefore, after a time, be absent, is not surprising. Moreover, in the duct ligation specimens, though there is considerable formation of scar tissue, the islets, which are apparently not diminished in numbers, and therefore under no physiological strain, present their normal granule content. Even by a neutral gentian stain, with which I have never succeeded in showing the specific granules in the graft islets of the dog, the granules of the duct ligation islets are easily seen. It is suggested, then, that when only a small portion of the pancreas is left, the characteristic granules of the B cells are exhausted. In a badly cicatrised specimen such cells are hard to distinguish, but there is reason to believe that more are present than are immediately evident, and it is possible that the ducts may even be forming islet tissue.

A consideration of the sugar function in connection with these changes shows that none of the animals became severely diabetic. A considerable amount of sugar appeared sooner or later in the urine of nearly all, and in the dog killed four days after operation it was present from the start. Less pancreas was left in this instance than in the other observations, probably too little to support life. Though the islet cells in this specimen were altered in the manner already noted, no conclusions can properly be drawn as to the relative importance of the acinous or islet tissue to carbohydrate metabolism.

The animal killed 16 days after operation was mildly diabetic from the start. Here the pancreatic acini were highly charged and in good condition, while the islets were composed of feebly stained shrunken cells. It would be reasonable to suppose that the latter were exhausted from carrying too heavy a load. The animal killed at the end of 26 days was not diabetic at first and never appeared at all ill. In this case, as in the 33-day graft, both acinous and islet tissue were apparently in process of destruction, and in both the characteristic appearance of normal islets was for the most part absent. It is unfortunate that the duct ligation experiment could not have been extended, but it is possible that some communication between the pancreas and bowel was being established.

Though a number of investigators have succeeded in reducing the pancreas to a condition in which no zymogenous tissue remained, and only islets were left, without bringing on diabetes, they have not been able to apply the final test of discovering whether the animal could live without what remained. This is true of the experiments of Laguesse, and others, on duct ligation in the rabbit, in which removal of the pancreas seems to be impossible. I believe also that no such final test has been applied to the dog with a satisfactory proof of the existence of only genuine islet tissue in what remained. Until such final proof is given, the association with the islets rather than the acini of an important sugar function is only implied. Nor does my incomplete investigation more than suggest this conclusion.

Conclusions.

1. The islets of Langerhans contain specific granules which allow of their positive identification.

2. There is no alteration in the islets, nor any evidence of conversion of acinous to islet tissue, under prolonged stimulation with secretin. On the contrary, the distinction between the two tissues is, under appropriate staining methods, more clear than usual.

3. There is no evidence of the conversion of acinous to islet tissue or the reverse, when only a small part of the pancreas is left to support life.

4. There is evidence that islet cells are reduced to a condition in which they appear similar to duct cells under these conditions, the first change being a disappearance or discharge of the granules characteristic of the B cells of Bensley.

5. There is evidence of an increase of duct tissue under the same conditions, but no evidence that this tissue produces new islets or takes up their function.

6. There is no positive evidence that islets are of vital importance to carbohydrate metabolism, but as between islet and acinous tissue the evidence favours the islet.

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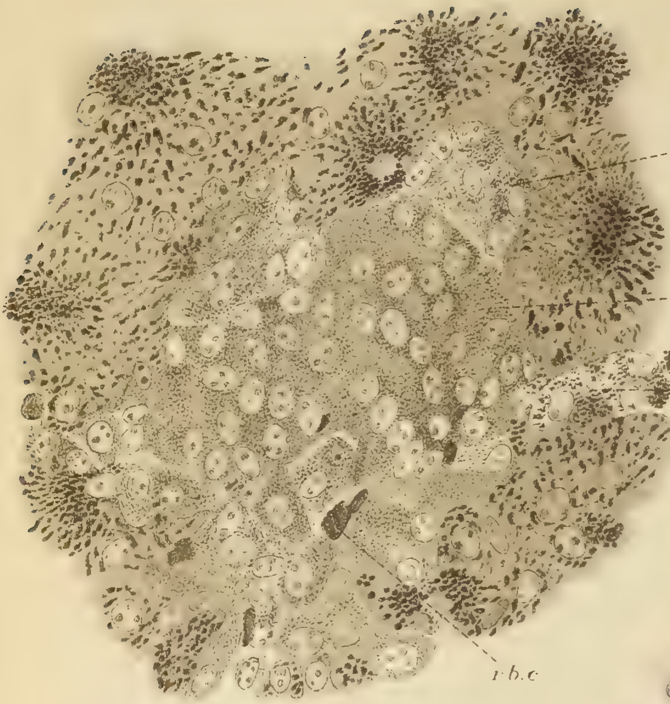

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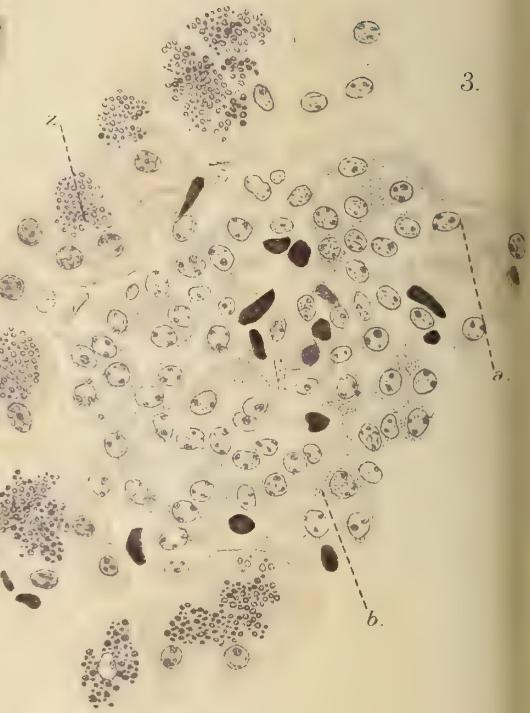

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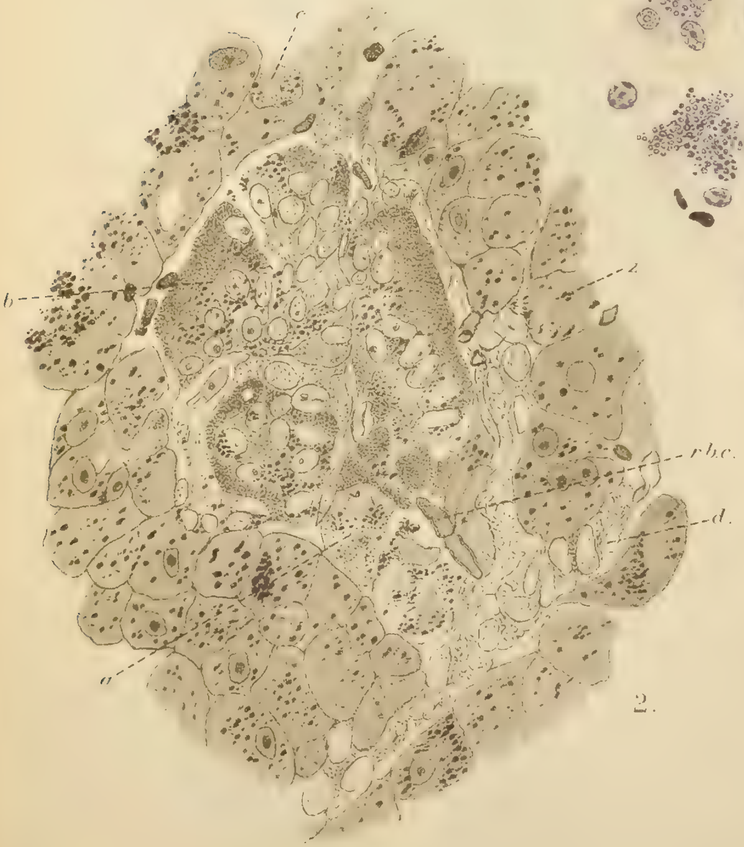

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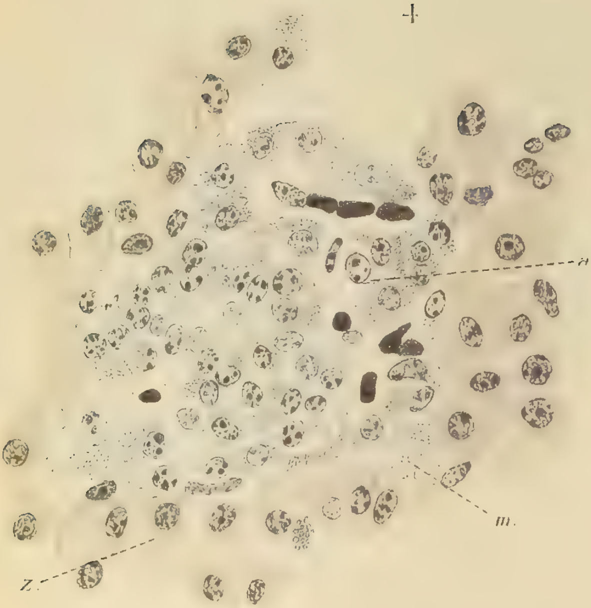

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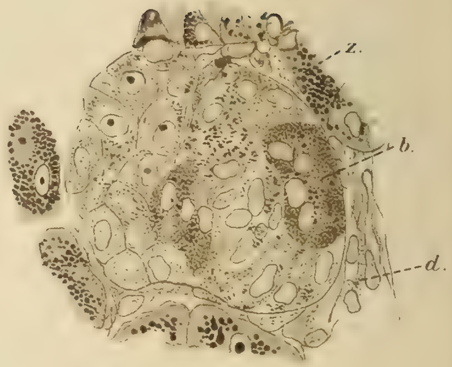

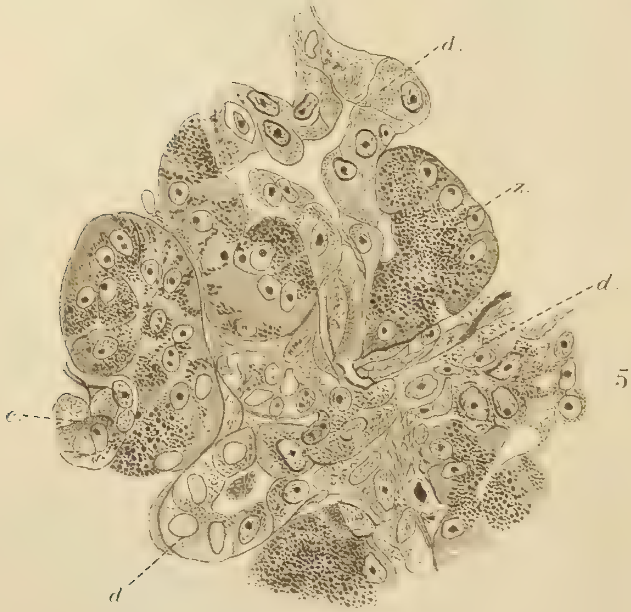

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DESCRIPTION OF PLATES 2 AND 3.

Fig. 1.—Normal islet in the charged pancreas of the dog. *a*, A cells of Lane. *b*, B cells of Lane. *c*, centroacinous cells. *r.b.c.*, red blood corpuscles. Notice the arrangement of the large round zymogen granules in the surrounding acini, also the distribution of the mitochondrial filaments. The borders of the islet are extremely irregular and no limiting membrane is present.

Fig. 2.—Normal islet in the exhausted pancreas of the dog. *a*, A cells of Lane. *b*, B cells of Lane. Notice the distribution of their granules along the capillaries, which are indicated by the red blood corpuscles (*r.b.c.*). *c*, centroacinous cells. *d*, duct cells, which are continuous with those of the islet below and low down on the right. *z*, exhausted acini. Notice the prevailing absence of zymogen granules, the presence of some mitochondrial filaments, and the vacuolisation of the cells. In the low right-hand corner a small group of acinous cells is cut off from the rest by a small duct.

Fig. 3.—Normal islet in the charged pancreas of the guinea-pig. *a*, A cells of Lane, undifferentially stained. *b*, B cells of Lane, containing fine blue granules. *z*, acini, containing zymogen granules.

Fig. 4.—Normal islet in the exhausted pancreas of the guinea-pig. *a*, A cells of Lane. *b*, B cells of Lane. *m*, acinous cells, containing "Mankowski" granules. *z*, acini without zymogen granules.

Fig. 5.—Group of duct cells and atrophied acini (from 26-day fragment). *c*, centroacinous cells. *d*, masses of duct cells, which partly retain their tubular arrangement and occupy the central part of the figure. Notice the resemblance of these cells in the arrangement of their mitochondrial filaments and granules to the islet of fig. 6.

Fig. 6.—Islet containing only a few typical B cells (from 26-day fragment). *b*, B cells of Lane. *d*, duct cells (poorly preserved). *z*, acini containing zymogen granules. Notice the small number of typical B cells. The rest of the islet cells might, from their appearance, be duct cells or A cells. Compare with figs. 5 and 1.

The Metabolism of Lactating Women.

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(Communicated by Dr. F. G. Hopkins, F.R.S. Received October 3, 1912,—Read January 23, 1913.)

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1. *Previous Work.*

The metabolic changes of pregnancy have been studied by various workers, principally from the point of view of comparing the total output of nitrogenous material with food intake.

The following results may be taken as proved:—

(1) There is a marked rise in the output of nitrogen following childbirth. Grammatikati (1), Zacharjewsky (2), and Slemons (3), among other workers, have definitely proved this.

(2) This increased nitrogen output more than counterbalances the nitrogenous intake, so that women, at this time, lose nitrogen. This is in marked contrast to the storage of nitrogen taking place before delivery.

The explanations offered by the various workers on these points vary considerably. Heinrichsen (4), Zacharjewsky (2), and Longridge (13) ascribe the increased nitrogen excretion to regressive changes in the puerperal woman, particularly changes affecting the uterus. Grammatikati (1) thought it was to be explained by mammary gland changes, more especially by the formation of milk fat from protein and the excretion of the nitrogenous residue.

One other point of interest observed by Slemons (3) is that the total nitrogen of the urine is less on the day of delivery than any other day and that the drop is greater the more prolonged the labour.

As for the individual nitrogenous substances excreted at this period but little is known. Ammonia, which forms a larger percentage of the total nitrogen with the advancement of pregnancy, gradually diminishes to the normal amount. Urea, as might be expected, forms the greater part of the increased nitrogenous excretion following childbirth and, according to Grammatikati, is a maximum when milk appears in the breast and diminishes with the weaning of the child. Other observers have not been able to corroborate this observation or to ascribe the causal connection between urea excretion and milk formation advanced by Grammatikati.

The appearance of creatin in the urine of lying-in women was first observed by Shaffer (5) and in dogs by Murlin (6). The present account is more particularly connected with this excretion of creatin by puerperal women. The analysis of such a condition seemed likely not only to furnish important results as to the life history of creatin, but also to shed light on the strange metabolic changes taking place in the body at this time.

2. *The Relation of the Puerperal Creatin Excretion to the Involution of the Uterus.*

Other well recognised conditions in which creatin is excreted include inanition and cancer of the liver (14), and since a striking feature about these conditions is the rapid wasting of the patient, it has been assumed that when the voluntary muscle breaks down, creatin is liberated into the blood-stream and excreted. This explanation was extended by Shaffer (5) to explain the puerperal excretion of creatin, but, in this case, the muscular tissue supposed to supply the creatin to the blood-stream was not the voluntary muscle but the involuntary muscle of the uterus. A serious difficulty, however, prevents the acceptance of this explanation, in that, while voluntary muscle contains abundant creatin, uterine muscle is quite devoid of this substance. In a previous paper (14), it was pointed out that creatin has a very limited distribution in nature and can only be found in the cross-striated muscle of vertebrate animals. The cross-striated muscle of invertebrates such as the lobster and the king crab, and, on the other hand, the smooth muscle of vertebrates, represent types of muscle which contain no creatin. Consequently, creatin cannot be found in the smooth, unstriated muscle of the uterus. It has been maintained that creatin and creatinin are present in tissues like the uterus because extracts of such tissues frequently give the Weyl or the Jaffé colour reactions. Both these colour tests are given by so many other substances that they are unreliable* as proofs of the presence of creatinin.

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* Weyl's colour test with sodium nitroprusside and caustic soda is also given by aldehyde, acetone, acetophenone, and aceto-acetic acid.

Jaffé's colour test with picric acid and soda is given by any reducing agent.

The red colour, which may be developed by mixing extracts of uterine muscle with alkaline picric acid, disappears rapidly on dilution, and cannot, therefore, be due to creatinin. Consequently, Shaffer's explanation, as it stands, does not adequately explain the puerperal excretion of creatin. However, it is possible that, although uterine muscle contains no creatin, yet some substance is present in the muscle which is converted into creatin when that tissue involutes, and appears ultimately in the urine. In order to test this point, the following observations were made.

In a lying-in ward two women were delivered of children by Cæsarian section, the one case (A) because of a contracted pelvis, the second case (B) because of a ruptured uterus. At the time of operation the uterus in Case A was stitched up and retained, while in Case B, with the ruptured uterus, it was completely removed. If the involution of the uterus is accountable for the *post-partum* excretion of creatin, then it is clear that Case A would excrete much more creatin than Case B, where there was no uterus to involute.

A. Cæsarian Section. Uterus stitched up and retained.
Operation January 13, 1910.

	Vol.	Total creatin.	Total creatinin.	$\frac{\text{Creatin}}{\text{Creatinin}}$.
	c.c.	grm.	grm.	
Jan. 14	815	0·987	1·03	0·96
„ 15	650	0·87	0·91	0·95
„ 16	450	0·58	0·54	1·08
„ 17	650	0·72	0·7	1·03
„ 18	1200	0·7	0·97	0·72
„ 19	1650	0·7	0·86	0·89

B. Cæsarian Section. Uterus removed.
Operation January 16, 1910.

	Vol.	Total creatin.	Total creatinin.	$\frac{\text{Creatin}}{\text{Creatinin}}$.
	c.c.	mgrm. in 10 c.c.	mgrm. in 10 c.c.	
Jan. 17	Spec.	9·7	10	0·97
„ 18				
„ 19 „ 20	2500	grm. 3·8	grm. 1·75	2·13
„ 21	1270	1·76	1·27	1·38

These figures show that the removal of the uterus did not prevent the excretion of creatin, and in fact, Case B, where there was no uterus to

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Fortunately, creatinin has a much more potent action than other physiological reducing agents and carries on the reaction very quickly with the formation of di-amino-mononitrophenol—a red substance which retains its intensity on strong dilution. The end product of such reducing agents as dextrose, levulose, maltose, aldehyde, is generally mono-aminodinitrophenol, which is also a red substance, but loses all colour intensity on dilution [Chapman (15)].

This explains why the full dilution of Folin's method of estimating creatinin is absolutely essential; for it is clear that on small dilution the colour of the mono-amino-dinitrophenol will interfere with that of diaminomononitrophenol. This point concerning dilution is a common source of error in recent research on creatinin.

involute, excreted much larger quantities of creatin. It would be unfair to press the interpretation of these experimental figures too far, because I think the excretion of creatin following a Cæsarian section may not be completely analogous to that accompanying a normal pregnancy. For instance, the following figures show that, even after an abdominal hysterectomy for uterine fibroids, creatin is excreted:—

Abdominal Hysterectomy. Fibroids of Uterus.

Time after operation.	Creatin in 10 c.c.	Creatinin in 10 c.c.	$\frac{\text{Creatin}}{\text{Creatinin}}$.
	mgrm.	mgrm.	
18 hours	9·9	14·0	0·7
4 days............	16·0	12·8	1·25
8 „	4·3	9·6	0·45

It may further be stated that all abdominal operations result in the excretion of some, but very variable amounts of creatin. The significance of this fact will be considered elsewhere.*

As regards the Cæsarian section figures two points are worthy of mention.

(1) Although such large amounts of creatin were excreted, the creatinin excretion was normal or but little diminished. In other words, the large creatin excretion was not produced at the expense of the creatinin. For instance, Case B excreted 2·86 grm. of (creatin + creatinin) per diem on an average over three days, whereas in normal health such a woman would excrete but little more than 1 grm. of creatinin and no creatin. This point is important, because it is commonly said that creatin is converted into creatinin, probably by the liver, and also that an increase of creatin excretion is accompanied by a diminution of creatinin. This relation may possibly hold in conditions like inanition, and in the absence of carbohydrate (Cathcart, 7) from the diet, for in such conditions there is usually a diminution of creatinin excreted, together with an increase of creatin. But it does not hold in the above cases of Cæsarian section.

(2) The creatin excretion following Cæsarian section did not depend on inanition or absence of carbohydrate from the diet. Both patients were taking an adequate amount of food throughout the period of examination and the urine did not indicate any condition of acidosis.

To sum up, there is no evidence that the puerperal creatin excretion depends, to any extent, on the involution of the uterus.

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* In the meantime I should like to utter a warning with regard to the interpretation of experiments on creatin metabolism which involve opening up the abdominal cavity.

3. *Evidence of the Relation between the Puerperal Excretion of Creatin and Mammary Gland Activity in Women.*

Pregnant rabbits, like pregnant women, excrete abnormal quantities of creatin. In fact the estimation of the creatin in the urine of a rabbit is a useful means of diagnosing pregnancy. It may be well to state that in my experience all rabbits, male and female, excrete small quantities of creatin. Oxen also, independently of sex differences, normally excrete creatin in addition to creatinin. This observation, affecting herbivorous animals, is in marked contrast to the total absence of creatin from the urine of normal people. When I started to investigate the puerperal excretion of creatin, rabbits seemed to be the best animals for examination. It was surprising, however, to find that the excretion of creatin by rabbits stops immediately after delivery, and consequently they were useless for this investigation. One possible factor suggested itself as an explanation of this difference between rabbits and human beings, namely, that rabbits eat their placentæ after delivery. It might be imagined that the proper performance of the functions of the organism after parturition depended upon the presence of certain chemical groupings, which were supplied by the digestion and assimilation of the placenta; but that, in the human being, since no placenta was available, the substances had to be supplied at the expense of other tissues such as the muscles, with the result that creatin was liberated and excreted at the time of the transference of material. If natural craving for animal food is any indication of physiological needs, then it is certain that female animals require the chemical substances of tissues such as muscle after parturition. The longing which such women have for meat has its analogy in the lower animals in their eating placentæ and frequently their young. In the case of herbivorous animals this seems to be the only time in their lives that they are carnivorous and may have some special significance such as the urgent requirements of the animal organism for substances such as extractives.

From this point of view, therefore, a cow was allowed to eat its placenta after parturition, in order to see whether the creatin excretion would be suppressed. The following figures were obtained:—

Cow.

	Creatinin in 10 c.c.	Creatin in 10 c.c.	$\frac{\text{Creatin}}{\text{Creatinin}}$
	mgrm.	mgrm.	
June 2	6·0	2·7	0·45
„ 3	8·0	2·6	0·325
, 4	10·1	4·1	0·405
„ 5	9·0	3·2	0·356
„ 6*	8·5	3·5	0·41
„ 7	6·6	5·3	0·8
„ 8	6·0	4·0	0·67
„ 9	6·2	3·0	0·48
„ 10	6·0	4·3	0·71

* Calf born June 6, 4 A.M. Placenta eaten by 10 A.M.

It will be seen that a rise of creatin excretion follows the ingestion of the placenta rather than a fall. Consequently it is unlikely that placenta eating explains the absence of creatin in a rabbit's urine *post partum.*

The investigation was continued on women and this will now be described.

(a) *A Quantitative Relation between Creatin Excretion and Milk Secretion in Nursing Women.*—The women were patients at a lying-in hospital,* and the diet in all cases was creatin-free, in that no meat, fish or meat extracts were given. Such a diet† has great drawbacks because of the desire, mentioned above, which these women have for meat and tasty food. The disappointment at the plainness of the diet often makes them depressed, and the mental condition is soon reflected in the poor progress made by the child, either because it is not properly nursed or because the mother's milk is of poorer quality. This difficulty was especially obvious in cases of multiparæ who had been in the lying-in hospital before and were therefore accustomed to plenty of meat. Most of the cases studied, therefore, were primiparæ, in whom this mental depression was not so obvious.

Below are the figures obtained in the examination of four normal cases of childbirth. They were all on the same diet and were living in the same ward under precisely the same conditions.

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* The Lambeth Lying-in Hospital, S.E., to the staff of which, and in particular to Dr. J. S. Fairbairn, I wish to express my indebtedness.

† A typical day's diet, though it varied to some extent, was as follows:—Milk 25 oz., gruel 5 oz., cocoa (made of milk) 5 oz., an egg, bread and butter 5 oz. Occasionally liver up to 4 oz. was added.

Mrs. St. C., delivered 10.35 A.M., December 9, 1910. Primipara.

	Vol.	N in 5 c.c.	Creatinin in 10 c.c.	Creatin in 10 c.c.	Total N.	Total creatinin.	Total creatin.	$\frac{\text{Creatin}}{\text{Creatinin}}$.	Baby's weight.
	c.c.	grm.	mgrm.	mgrm.	grm.	grm.	grm.		oz.
Before delivery	Spec.	—	5·2	1·2	—	—	—	0·23	—
1st day, up to 6 P.M., Dec. 9 ...	700	0·045	5·6	1·4	6·25	0·393	0·1	0·25	80
2nd " " 10 ...	1330	0·039	7·4	1·2	10·5	0·985	0·16	0·162	77
3rd " " 11 ...	970	0·044	9·2	1·4	8·44	0·891	0·136	0·152	75
4th " " 12 ...	1510	0·039	6·8	2·1	11·8	1·03	0·317	0·310	77
5th " " 13 ...	1000	0·049	8·1	2·8	9·75	0·81	0·28	0·345	77
6th " " 14 ...	490 (?)	0·056	9·6	2·7	5·5	0·473	0·133	0·282	78
7th " " 15 ...	980	0·057	9·9	3·6	11·3	0·97	0·353	0·363	79½
8th " " 16 ...	340 (?)	0·068	11·0	3·8	4·67	0·374	0·129	0·345	79
9th " " 17 ...	830	0·052	9·4	3·7	8·64	0·783	0·308	0·394	79½

$\frac{\text{Creatin}}{\text{Creatinin}}$ ratio (after establishment of lactation) = 0·340. Gain in weight of baby during same period = 4½ oz. = 0·75 oz. per diem.

Mrs. Gr., delivered 2.25 P.M., May 25, 1910. Primipara. Baby's weight at birth, 120¾ oz.

	Vol.	N in 5 c.c.	Creatinin in 10 c.c.	Creatin in 10 c.c.	Total N.	Total creatinin.	Total creatin.	$\frac{\text{Creatin}}{\text{Creatinin}}$.	Baby's weight.
	c.c.	grm.	mgrm.	mgrm.	grm.	grm.	grm.		oz.
Before birth	Spec.	—	4·9	trace	—	—	trace	0·0	—
1st day, up to 6 P.M., May 26...	1110	0·026	7·0	trace	5·8	0·777	trace	0·0	114½
2nd " " 27...	1060	0·062	13·3	trace	13·2	1·41	trace	0·0	111½
3rd " " 28...	670	0·085	15·0	7·0	11·5	1·01	0·469	0·47	114
4th " " 29...	450 (?)	0·087	11·6	5·9	7·9	0·52	0·265	0·51	116½
5th " " 30...	980	0·091	13·5	6·8	17·8	1·32	0·666	0·50	116¾
6th " " 31...	910	0·089	12·4	6·6	16·3	1·13	0·600	0·53	117½
7th day, up to 6 P.M., June 1...	830	0·09	13·3	3·9	14·9	1·10	0·324	0·29	117½
8th " " 2...	930	0·085	11·0	4·2	15·8	1·02	0·391	0·38	117½
9th " " 3...	800	0·09	14·0	4·3	14·4	1·12	0·344	0·31	118

$\frac{\text{Creatin}}{\text{Creatinin}}$ ratio (after establishment of lactation) = 0·427. Increase in baby's weight during corresponding period = 6·5 oz. = 0·93 oz. per diem.

Mrs. Gy., delivered 3.25 P.M., May 24, 1910. Primipara. Weight of baby at birth, 115¾ oz.

	Vol.	N in 5 c.c.	Creatinin in 10 c.c.	Creatin in 10 c.c.	Total N.	Total creatinin.	Total creatin.	$\frac{\text{Creatin}}{\text{Creatinin}}$.	Baby's weight.
	c.c.	grm.	mgrm.	mgrm.	grm.	grm.	grm.		oz.
1st day, up to 6 P.M., May 25...	1230	0·024	5·5	1·1	6·0	0·675	0·135	0·2	109½
2nd ,, ,, 26...	690	0·049	8·3	4·6	6·8	0·575	0·317	0·552	106½
3rd ,, ,, 27...	560	0·079	10·9	5·8	8·8	0·61	0·325	0·487	108½
4th ,, ,, 28...	700	0·083	8·5	4·5	11·6	0·595	0·315	0·528	109½
5th ,, ,, 29...	780	0·084	8·6	5·6	13·1	0·67	0·437	0·650	112
6th ,, ,, 30...	740	0·094	10·0	6·9	13·9	0·74	0·51	0·690	113
7th ,, ,, 31...	1070	0·067	7·5	4·1	14·2	0·80	0·435	0·547	114½
8th day, up to 6 P.M., June 1...	1020	0·056	6·1	2·3	11·5	0·63	0·235	0·377	114½
9th ,, ,, 2...	570	0·083	10·4	4·4	9·5	0·59	0·25	0·417	115

$\frac{\text{Creatin}}{\text{Creatinin}}$ ratio (after establishment of lactation) = 0·531. Gain in weight of baby during same period = 8½ cz. = 1·21 oz. per diem.

Mrs. T., delivered 12.55 A.M., May 26, 1910. Multipara (third). Baby's weight at birth, 124½ oz.

	Vol.	N in 5 c.c.	Creatinin in 10 c.c.	Creatin in 10 c.c.	Total N.	Total creatinin.	Total creatin.	$\frac{\text{Creatin}}{\text{Creatinin}}$.	Baby's weight.
	c.c.	grm.	mgrm.	mgrm.	grm.	grm.	grm.		oz.
Before birth	—	—	8	2·3	—	—	—	0·29	—
1st day, up to 6 P.M., May 27...	910	0·031	6·75	2·0	5·6	0·615	0·182	0·27	119
2nd ,, ,, 28...	1100	0·051	8·0	4·6	11·2	0·88	0·51	0·57	120
3rd ,, ,, 29...	700	0·088	12·2	8·5	12·4	0·86	0·595	0·68	123
4th ,, ,, 30...	1280	0·053	6·5	4·1	13·5	0·83	0·51	0·63	128
5th ,, ,, 31...	970	0·055	7·4	3·95	10·7	0·72	0·385	0·53	130½
6th day, up to 6 P.M., June 1...	1350	0·046	6·2	4·4	12·4	0·84	0·595	0·71	130½
7th ,, ,, 2...	1490	0·041	4·9	3·2	12·1	0·73	0·477	0·65	131
8th ,, ,, 3...	1040	0·051	6·9	5·1	10·7	0·72	0·55	0·77	131½
9th ,, ,, 4...	1050	0·050	7·2	3·7	10·5	0·76	0·39	0·51	132⅓

$\frac{\text{Creatin}}{\text{Creatinin}}$ ratio (after establishment of lactation) = 0·631. Gain in weight of baby during corresponding period = 13·5 oz. = 1·70 oz. per diem.

The following points are indicated by these figures:—

(1) There is a rapid increase in the nitrogen excreted in the first few days after delivery. This point has been frequently observed before and has been commented upon at the beginning of this paper. It will be noted that the rise usually starts on the second day, but in the case of Mrs. Gy. it is delayed until the fourth day. This variation in different women has also been previously noticed and has not been interpreted satisfactorily, some ascribing it to variations in uterine involution, others to variations in mammary gland activity.

(2) Except possibly for the first day after delivery the excretion of creatinin exhibits its usual constancy.*

(3) The creatin rises in the first few days. Consequently, there is a rise in the $\frac{\text{creatin}}{\text{creatinin}}$ ratio in these days.

(4) An examination of the $\frac{\text{creatin}}{\text{creatinin}}$ ratio of the four cases and a comparison with the rates of progress of the infants indicate that the increase in weight of the children is roughly proportional to the creatin excreted by the respective mothers, thus:

	$\frac{\text{Creatin}}{\text{Creatinin}}$.	Increase of baby's weight per diem.
		oz.
Mrs. St. C.	0·340	0·75 (Baby St. C.)
„ Gr.	0·427	0·93 (Baby Gr.)
„ Gy.	0·531	1·21 (Baby Gy.)
„ T.	0·631	1·70 (Baby T.)

It may be stated that the children were entirely breast-fed, so that whatever increase in weight they experienced was due to the milk secreted by the mother's mammary glands. Consequently, the above figures indicate that the $\frac{\text{creatin}}{\text{creatinin}}$ ratio of the urine is related either to the quantity or quality, or both, of the milk secreted by the mammary glands of the mother. In keeping also with this interpretation is the fact that the $\frac{\text{creatin}}{\text{creatinin}}$ ratio increases *pari passu* with mammary gland activity in the first two days after delivery, at a time when the colostrum is being changed to milk.

It is evident that, in order to establish the hypothesis of a relation between creatin excretion and mammary gland activity, other evidence, in addition to that offered, is necessary. It would be advantageous if, for instance, it were possible to calculate directly the amount of milk secreted by a nursing woman. This was not found to be possible. The following indirect evidence, however, may now be considered:—

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* This constancy is such that when there is any great diminution in a day it may usually be assumed that the 24 hours' specimen is not complete.

(b) *A Case of Creatin Excretion and Mammary Gland Activity developing late after Childbirth.*—The next point to be observed is that when a woman's breasts have their activity delayed, following childbirth, then there is a corresponding delay in the creatin excretion. This patient* may be described as a case suffering from a toxæmia of pregnancy, and showed the characteristic symptoms of this condition, namely, general œdema, headache and occasional temporary attacks of blindness. She also excreted much albumen. She never had any eclamptic fits; and, although very ill, labour was not induced, but came on naturally at the end of the eighth month. At the birth of the child the breasts were soft and without any trace of activity. They became gradually active about the fourth day after delivery, and at this time also creatin, which had been, up till this time, quite absent, began to appear and increase.

Mrs. T. (toxæmia of pregnancy). Weight of baby, 3½ lb. Eighth month.

Date.	$\frac{\text{Creatin}}{\text{Creatinin}}$.	Albumen (by Esbach), approximate.
		grm.
February 2	Trace of creatin	—
„ 7	„ „	—
„ 12	„ „	7·7
„ 15	„ „	4·77
„ 16*	„ „	5·17
„ 17	„ „	4·03
„ 18	„ „	4·6
„ 19	0·15	0·66
„ 20	0·21	1·9
„ 21	0·35	0·46
„ 22	0·36	0·0
„ 24	0·57	0·0
March 2	0·3	
„ 5	0·18	
„ 8	0·16	

* Time of delivery, February 16, 10 A.M.

In this case the creatin excreted was too small to be estimated until the third day after delivery.

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* The case was met with in an investigation of the toxæmias of pregnancy, undertaken in conjunction with Dr. J. P. Hedley.

On February 18, 5 drops of colostrum were squeezed from the breasts.
19, ½ oz. colostrum was withdrawn in the morning. By
6 P.M. 1 oz. could be withdrawn every two hours.
20, lactation established.

As this baby was too feeble to suck, the milk had to be first withdrawn from breasts.

It is interesting to compare the creatin excreted by this toxæmic patient with that of a normal case of pregnancy where labour came on prematurely at the eighth month. She was in good health, and nursed her baby from the first.

Mrs. B. (normal case). Premature labour. About eighth month. Weight of baby, 5¼ lb.

Date.	$\frac{\text{Creatin}}{\text{Creatinin}}$.
February 7	0·29
„ 14*	—
„ 15	0·22
„ 16	0·31
„ 17	0·34
„ 18	0·78
„ 19	0·49
„ 21	0·27
„ 23	0·28

* Baby born February 14.

In this case the creatin excretion was always present, and increased, as after the birth of normal full-term children, for a few days. This is in marked contrast to the case of Mrs. T., where the creatin is absent before delivery, and until the breasts become active. Another interesting point about this toxæmic case is that the appearance of creatin synchronises with a large decrease of albumen in the urine. It appears as if the cause of the albuminuria was also the cause of the suppressed creatin excretion. A similar case of toxæmia of pregnancy has been met with.

(c) *A Case Illustrating Simultaneously Suppressed Creatin Excretion and Mammary Gland Activity.*—Another piece of evidence that creatin excretion and mammary gland activity are related is as follows: if a condition arises, in the first few days after milk secretion is fully established, to suppress the milk secretion, then the creatin excretion stops at the same time.

In the following case, N. was delivered of a baby on May 25. The milk was abundant, and the child developed very well until June 1, when the left breast developed an abscess, and the temperature rose to 102°. This

Mrs. N., delivered 12.15 A.M., May 25, 1910. Primipara. Weight of baby at birth, 106 oz.

	Vol.	N in 5 c.c.	Creatinin in 10 c.c.	Creatin in 10 c.c.	Total N.	Total creatinin.	Total creatin.	$\frac{\text{Creatin}}{\text{Creatinin}}$	Baby's weight.
	c.c.	grm.	mgrm.	mgrm.	grm.	grm.	grm.		oz.
Before labour	Spec.	—	6·8	2·3	—	—	—	0·34	—
1st day, up to 6 P.M., May 25	242	—	4·65	1·2	14·1	1·13	0·292	0·26	104¾
2nd " " 26	680	0·077	10·0	6·1	10·5	0·68	0·415	0·61	99½
3rd " " 27	960	0·072	9·0	5·4	13·8	0·865	0·52	0·60	99½
4th " " 28	690	0·076	9·0	4·2	10·5	0·62	0·29	0·47	102
5th " " 29	880	0·08	9·4	3·7	14·1	0·83	0·325	0·39	103
6th " " 30	790	0·088	9·3	5·4	14·0	0·74	0·43	0·58	101
7th " " 31	950	—	—	—	—	—	—	—	102½
8th day, up to 6 P.M., June 1*	Spec.	—	10·0	2·2	—	—	—	0·22	103
9th " " 2	620	0·084	10·8	1·0	10·4	0·67	0·062	0·092	101
10th " " 3	650	0·089	11·9	1·0	11·6	0·77	0·065	0·084	103
11th " " 4	560	0·083	11·6	0·0	9·3	0·65	0·0	0·0	104½

* Temperature up to 102°, due to mammary gland abscesses. Milk secretion was suppressed, and from this point the baby was hand fed. Note the suppression of the creatin with the development of the temperature.

was followed by abscesses in the right breast. Both breasts were too painful for nursing the child, and, as the result of the abscesses and hyperpyrexia, the secretion of milk was rapidly and totally suppressed.

It might be supposed that, since in this case the creatin excretion and the milk secretion were synchronously suppressed, any case of suppression of milk secretion, produced by the ordinary methods of banding and purgation, would also cease to excrete creatin. This is not the case, as the following figures show:—

M 18. Confined September 5, 1911. Prolapse of cord, still-born child, placenta prævia. Had nursed previous children. Treatment:—Breasts bandaged tightly, two or three purges each day.

	$\frac{\text{Creatin}}{\text{Creatinin}}$.
September 12	0·79
„ 14	0·62
„ 15	0·78
„ 16	0·70

It will be seen that not only is the creatin not suppressed in this case, but is higher than the normal cases of nursing women given above. If, then, the mammary glands in such a case were without milk, and completely flaccid, it would disprove the relation of creatin excretion and mammary gland activity which it has been the object of this paper to establish. In point of fact, on September 16, the breasts of this woman, in spite of all the purgation she had experienced, contained abundant milk, and were hard and knotty.

On September 8 she had a temperature of 100·2°, due, no doubt, to the congestion of the mammary glands. There is no doubt that purgation and breast bandaging do not suppress mammary gland activity in the sense that illness, particularly when there is fever, does. A feverish condition seems to produce very quickly soft, flabby breasts, markedly different from the hard, knotty breasts of a woman treated by purgation. In keeping with this is the fact that in the one case the excretion of creatin is diminished, and, in the other, is as high, or higher, than normal.

4. *The Effect of Adding Casein to the Diet of a Puerperal Woman.*

Having got some evidence of a relation between mammary gland activity and creatin excretion, the investigation was continued in order to determine whether there is any obvious relation between particular branches of the metabolism of secreting breasts and creatin.

It seemed possible that the *post-partum* excretion of creatin might depend on the metabolic changes taking place in the body which culminate in the formation of caseinogen in the mammary gland at this period. It can be well imagined that the demand of the mammary gland for specific chemical groupings necessary for the formation of caseinogen itself might result in chemical changes, particularly in the muscle, which would cause the liberation of creatin into the blood-stream and its subsequent excretion. If such were the case, then the addition of casein to the diet might render unnecessary the creatin-liberating changes and effect a corresponding disappearance of excreted creatin at this time.

In the two following cases, 50 grm. of casein were added to the creatin-free diet each day.

It is evident that there is no diminution of creatin excreted as the result of casein feeding, and the results lend no support to the hypothesis that the metabolic changes involved in the formation of caseinogen by the mammary gland are related to the *post-partum* excretion of creatin.

5. *The Independence of the Puerperal Excretion of Creatin and Carbohydrate Metabolism.*

The *post-partum* excretion of creatin is a good example of the fact that there may not be anything, so far as is known, wrong or abnormal with carbohydrate in the body while at the same time large quantities of creatin are being excreted.

Needless to say there is no acidosis in the case of a normal pregnancy, so any relation between creatin excretion and carbohydrate is not so obvious as such a condition would signify. For it is probable that all cases of acidosis are accompanied by the excretion of some, although widely variable amounts of, creatin. It seemed likely that the carbohydrate abnormality responsible for the creatin excretion at this period was not an absolute deficiency of carbohydrate in the body, but rather a sidetracking of certain constituents of the carbohydrate in order to furnish an adequate supply of lactose-forming substances to be dealt with by the mammary gland. Consequently in the two cases now to be described lactose and dextrose were respectively added to their otherwise abundant diets in order to satisfy any glycogen and dextrose deficiency of the liver, and also the lactose requirements of the mammary gland.

It is obvious from these two carbohydrate-feeding experiments that if there is any connection between carbohydrate and creatin excretion during puerperium, it is obscure and of such a nature that alimentary carbohydrate plays no part in the relation. In neither case—lactose feeding and

Mrs. M. Primipara. Baby born 3.40 P.M., January 15, 1911.

	Vol.	N in 10 c.c.	Creatinin in 10 c.c.	Creatin in 10 c.c.	Total N.	Total creatinin.	Total creatin.	$\frac{\text{Creatin}}{\text{Creatinin}}$.	Weight of baby.
	c.c.	grm.	mgrm.	mgrm.	grm.	grm.	grm.		oz.
1st day, up to 6 P.M., Jan. 16...	1250	0·052	4·5	1·51	6·54	0·564	0·189	0·336	100
*2nd ,, ,, 17...	1180	0·070	5·5	2·56	7·93	0·62	0·288	0·465	98½
*3rd ,, ,, 18...	810	0·091	6·3	3·14	8·06	0·555	0·276	0·498	98
*4th ,, ,, 19...	1680	0·087	4·8	2·44	14·7	0·808	0·41	0·51	99
*5th ,, ,, 20...	910	0·107	5·4	1·99	9·75	0·493	0·181	0·368	99½
*6th ,, ,, 21...	1150	0·172	7·7	3·25	19·8	0·886	0·373	0·422	102½
*7th ,, ,, 22...	1760	0·098	4·3	1·86	17·3	0·757	0·327	0·433	102½
*8th ,, ,, 23...	1200	0·132	5·8	3·72	15·9	0·696	0·446	0·64	102½
*9th ,, ,, 24...	670	0·113	4·9	2·21	7·6	0·328	0·148	0·45	104
*10th ,, ,, 25...	1240	0·083	3·4	2·20	10·3	0·423	0·273	0·65	105½
*11th ,, ,, 26...	670	0·158	6·9	3·25	10·6	0·464	0·218	0·472	107

Baby entirely breast fed. $\frac{\text{Creatin}}{\text{Creatinin}}$ (from time of establishment of lactation) = 0·493. Increase of weight of baby per diem = 1·12 oz.

* 50 grm. of casein to mother, in addition to creatin-free diet.

Mrs. A. Primipara. Baby born 6.55 A.M., January 17, 1911.

	Vol.	N in 10 c.c.	Creatinin in 10 c.c.	Creatin in 10 c.c.	Total N.	Total creatinin.	Total creatin.	$\frac{\text{Creatin}}{\text{Creatinin}}$.	Weight of baby.
	c.c.	grm.	mgrm.	mgrm.	grm.	grm.	grm.		oz.
1st day, up to 6 P.M., Jan. 17...	250	—	7·5	1·28	—	0·184	0·032	0·17	100
*2nd ,, ,, 18...	1170	0·09	8·0	1·86	10·5	0·94	0·217	0·232	96
*3rd ,, ,, 19...	570	0·142	9·6	4·65	8·1	0·55	0·265	0·483	93½
*4th ,, ,, 20...	900	0·112	7·2	4·07	10·1	0·65	0·367	0·563	91
*5th ,, ,, 21...	860	0·113	6·0	3·83	9·74	0·517	0·33	0·638	93½
*6th ,, ,, 22...	1160	0·131	6·3	3·60	15·2	0·73	0·418	0·572	94
*7th ,, ,, 23...	810	0·136	7·2	3·95	11·0	0·584	0·32	0·55	95½
*8th ,, ,, 24...	640	0·153	7·4	4·3	9·8	0·475	0·275	0·58	97
*9th ,, ,, 25...	—	0·159	8·0	5·1	—	—	—	0·64	98½
*10th ,, ,, 26...	1390	0·087	5·9	3·02	12·1	0·82	0·42	0·51	96½†
11th ,, 6 A.M. ,, 27... (12 hrs.)	380	—	6·8	2·44	—	—	—	0·36	98½

$\frac{\text{Creatin}}{\text{Creatinin}}$ ratio (after establishment of lactation) = 0·55. Increase in weight of baby = 7½ oz. in 7 days = 1·07 oz. per diem.

* On these days 50 grm. of casein added to diet.

† The loss in weight on this day may probably be accounted for by the development of whitlows on thumb and first finger of the mother, which had to be treated.

Mrs. N., delivered 2 P.M., December 8, 1910. Multipara (two).

	Vol.	N in 10 c.c.	Creatinin in 10 c.c.	Creatin in 10 c.c.	Total N.	Total creatinin.	Total creatin.	$\frac{\text{Creatin}}{\text{Creatinin}}$.	Baby's weight.
	c.c.	grm.	mgrm.	mgrm.	grm.	grm.	grm.		oz.
Before birth	Spec.	—	8·4	2·5	—	—	—	0·297	—
1st day, up to 6 P.M., Dec. 9...	1020	0·077	7·7	3·1	7·88	0·787	0·317	0·403	107
*2nd ,, ,, 10...	Spec.	—	11·4	5·1	—	—	—	0·447	102½
*3rd ,, ,, 11...	1030	0·13	11·1	6·5	13·4	1·15	0·673	0·585	104½
*4th ,, ,, 12...	1170	0·112	7·4	4·9	13·1	0·867	0·575	0·663	108
*5th ,, ,, 13...	360 (?)	0·173	11·4	7·7	6·28	0·411	0·277	0·675	111
*6th ,, ,, 14...	550	0·147	9·6	5·6	8·1	0·528	0·308	0·582	112
*7th ,, ,, 15...	840	0·149	9·6	4·3	12·6	0·808	0·362	0·447	114½
†8th ,, ,, 16...	690	0·132	9·6	2·7	9·14	0·664	0·186	0·28	113½
†9th ,, ,, 17...	810	0·138	9·7	5·2	11·2	0·787	0·422	0·536	113

During lactose period $\frac{\text{creatin}}{\text{creatinin}}$ ratio = 0·566, and increased weight of baby 1·57 oz. per diem. The child was entirely breast fed.

* On these days 50 grm. of lactose were added to diet. † On these days 75 grm. of glucose were added to diet.

Mrs. Str., delivered 6.23 A.M., December 9, 1910. Multipara (three).

	Vol.	N in 10 c.c.	Creatinin in 10 c.c.	Creatin in 10 c.c.	Total N.	Total creatinin.	Total creatin.	$\frac{\text{Creatin}}{\text{Creatinin}}$.	Baby's weight.
	c.c.	grm.	mgrm.	mgrm.	grm.	grm.	grm.		oz.
1st day, up to 6 P.M., Dec. 9...	Spec.	0·0477	3·4	1·05	—	—	—	0·307	106
*2nd ,, ,, 10...	1730	0·055	6·1	1·51	9·53	1·05	0·262	0·247	103
*3rd ,, ,, 11...	960	0·112	10·5	2·56	10·7	1·01	0·246	0·243	96½
*4th ,, ,, 12...	700	0·115	9·0	3·26	8·07	0·63	0·228	0·362	96½
*5th ,, ,, 13...	480	0·084	7·2	2·56	4·83	0·346	0·123	0·355	97½
*6th ,, ,, 14...	740	0·0695	5·8	2·21	5·15	0·43	0·163	0·380	99
*7th ,, ,, 15...	1870	0·0808	5·6	1·86	15·1	1·05	0·348	0·332	97½
8th ,, ,, 16...	810	0·109	7·0	2·32	8·84	0·568	0·188	0·332	97½
9th ,, ,, 17...	1050	0·113	7·6	3·6	11·9	0·798	0·377	0·474	96

$\frac{\text{Creatin}}{\text{Creatinin}} = 0·334.$ Baby lost weight.

* On these days 75 grm. of glucose (20 grm. to ℥1) were taken by the mother, in addition to ordinary food. The baby was breast fed until December 13, at which time, in consequence of poor progress, alternate feeds of cow's milk and cream were added to diet.

glucose feeding—was the creatin excretion abnormally low, and indeed in the former case the creatin ratio was high.

Another point worthy of comment is the very poor progress of the baby whose mother took glucose. Of all the cases investigated this baby did the worst.* This may have been a coincidence, but there is some evidence that the glucose solution itself had a detrimental effect, in that when it was substituted for lactose in the first case described, this baby's weight began to diminish, although previously it had developed remarkably well. On the two glucose-feeding days it lost 1½ oz. in weight, whereas in the previous two lactose-feeding days, the weight of the baby increased 3½ oz.

Whether or no glucose feeding to a puerperal mother has a detrimental effect in all cases on the development of an adequate mammary gland secretion, the above results quite suffice to demonstrate that the creatin excretion of the puerperium is of a different nature from that excreted during periods of inanition or when carbohydrate is withheld from the diet.

In view of the great amount of attention the relation of creatin to carbohydrate metabolism has in recent years attracted, it may be well to consider briefly this subject with reference to the results obtained in this section. It is generally admitted that if carbohydrate metabolism be abnormal in mammalia, then creatin is excreted. For instance, it has been shown that the creatin excretion produced by inanition can be cleared up by the ingestion of carbohydrate (Cathcart (7), Mendel and Rose (8)). Further, the absence of carbohydrate from an otherwise normal diet results in the excretion of creatin. Also in diabetes mellitus and phloridzin glycosuria (Krause and Cramer (9), Cathcart and Taylor (10)) creatin is excreted. It is natural that such facts have led many physiologists to assign the most intimate relationship between creatin and carbohydrate. For instance, it has been suggested that creatin forms compounds with carbohydrate in the liver, and is transported in such a combination to the muscles. Mendel and Rose (8) have stated that, "without question, the metabolism of creatin is intimately related with carbohydrate metabolism." Now it would appear that all the conditions studied in this connection, either pathological or the result of treatment, have one factor in common, namely, the carbohydrate metabolism is either known to be or is rendered abnormal, and then the creatin excretion is studied. But I venture to suggest that this is not sufficient to establish a direct relation between creatin and carbohydrate. If, as the result of recent metabolic research, one point has become more prominently emphasised than any other, it is that carbohydrates play a more important part than as simple sources of available energy. Incidentally, it may be mentioned that, even as sources of energy, the work of Landergren (11) made it clear that the body preferred carbohydrate to other substances. The excretion of β-oxybutyric acid and allied bodies in abnormal carbohydrate conditions makes it apparent that carbohydrate is necessary for the adequate performance of katabolic changes of fatty acids. Further, evidence has accumulated, since Lüthje (12) first advocated the theory, to show that carbohydrate is necessary for the synthesis of proteins from the amino-acids by the bioplasm of the animal.

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* The baby of the glucose-fed mother lost ground so rapidly that artificial feeding was added after the third day, but in spite of this the baby gained no weight so long as the investigation lasted.

Again, there is abundant evidence from the number of toxic substances, such, for instance, as phenol and camphor, which are excreted by the body in combination with glycuronic acid, that the poison neutralising powers of the body are largely dependent upon carbohydrates. In support of this fact, also, may be mentioned the experiments of Hildebrandt (16), who demonstrated the innocuous effects of an otherwise lethal dose of thymotin piperidid, if administered with dextrose or cane sugar.

These few instances show the importance of carbohydrate in physiological activity, and it would appear that nearly all the metabolic processes of the body, with which we are familiar, become abnormal both when it is absent and when it cannot be used. It is not, therefore, contrary to expectation if creatin metabolism, which is a comparatively recent innovation in biochemical development, and not even constant in different mammals, shows signs of abnormality when the carbohydrate stores become unavailable. But it seems to me that evidence of a different nature to that already adduced must be established before creatin can be as directly related to carbohydrate in the body as some would have us believe.

As regards fatty acid metabolism, it is generally agreed that not only does deficiency of carbohydrate in man necessitate the excretion of β-oxybutyric acid, but also the excretion of this latter substance is a reliable indication of abnormal carbohydrate metabolism. Can this test be applied to creatin and carbohydrate? It is an undoubted fact that abnormal carbohydrate metabolism is accompanied by an excretion of creatin. Does the excretion of creatin mean deficient available carbohydrate? The studies of creatin excretion by puerperal women, above described, lend no support to the view that carbohydrate is not abundant and available. Again, it is impossible to stop herbivorous animals like rabbits and cattle from excreting small quantities of creatin, no matter how much carbohydrate is eaten. Finally, it may be mentioned that the creatinuria in a case of cyclic vomiting recently described (17) could not be cleared up by feeding with carbohydrate. Such facts as these render it improbable that creatin excretion is, in any sense, an indication of abnormal carbohydrate metabolism, and it seems to be that the causal relationship commonly held to have been established between these two substances is quite unjustified. The situation can be summed up by the statement that creatin metabolism, together with many other biochemical changes in the organism, goes wrong in the absence of carbohydrate, but, on the other hand, an abnormal creatin metabolism does not mean an abnormal carbohydrate metabolism.

6. *General Considerations.*

There is some evidence that the puerperal excretion of creatin depends upon the action of a substance formed in the mammary gland itself. For instance, it was seen above that the woman who did not suckle her child after parturition excreted an abnormally large proportion of creatin. In such a case, when the mammary gland is not freed of its milk in the normal way, any active substance would undoubtedly be absorbed to excess into the blood-stream from the gland, and an excessive excretion of creatin result.

Just as the ovum at an early stage of development must produce some potent physiological substance in order to alter the disposition of nutriment in the maternal organism for its own benefit, so the mammary gland, after childbirth, must use equally powerful means to force its claim upon a community of cells, each fighting for the good things distributed by the blood. The potency of the substances formed in the early fœtus is evident in the morning sickness and other unpleasant symptoms of pregnancy, while, on the other hand, there is evidence of a similar nature that the mammary glands immediately after childbirth contain physiologically active substances. For instance, the milk fever of cows is a condition for which the mammary glands are largely responsible.* That this is so is evident by the treatment of the condition, a treatment which generally brings about recovery, namely, to inflate the udders with air. Such treatment suppresses the activity of the mammary gland, and, no doubt, stops the formation of the active toxic substances.

Somewhat analogous to the milk fever of cows is the very common pyrexia seen in women about the third day after childbirth, more especially in those women where the milk secretion is very abundant and the breasts are congested and tender. It is only by careful evacuation of the breasts that pyrexia is avoided at these times, and the cause of the fever is undoubtedly some substance formed in the mammary gland and absorbed in excess into the general circulation. It may be that the substance formed in the mammary gland and responsible for the pyrexia of the puerperium may also account for the creatin excretion at this period and the great metabolic changes which result in the transference of nutrient material to the breasts. Another hypothesis which links up the same facts is that toxic substances may be formed as waste products in the mammary gland and ovum at early stages of development and that these substances cause the excretion of creatin. Certainly, in several cases where there was slight pyrexia after parturition, associated with mammary gland congestion, I found a high $\frac{\text{creatin}}{\text{creatinin}}$ ratio in the urine. Such an explanation, also, would fall into line with the fact that the best milking mothers excreted the most creatin.

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* Dr. Pembrey informs me that another important factor in milk fever is the state of nutrition of the cows. Pregnant cows which have been overfed are much more liable to milk fever.

The fact that creatin is also excreted during pregnancy indicates that there is nothing specific about the probable relation of creatin excretion and mammary gland activity. In the adult person it seems to be a general reaction indicating some abnormal condition affecting the transport of nutrient material, such, for instance, as that which must accompany the growth of a fœtus* or the development of milk secretion. Such a generalisation would include the metabolic condition of carbohydrate deficiency, where, again, creatinuria is present. Also, the results of Mendel and Rose (18), who observed the excretion of creatin in all stages of childhood and adolescence, suggest the association of creatin with the transport of material necessary for the growing tissues.

In most of the other conditions brought to light in which large quantities of creatin are excreted, it has been possible to show that the liver is primarily affected: such conditions for instance as cancer of the liver, deficiency or abnormality of carbohydrate metabolism, toxæmias directly affecting the liver as when a septic focus is in the abdominal cavity, and after operative interference with parts of the body supplied by the portal circulation.† It has not been possible to get any evidence of liver abnormality in the case of lactating women, but, by analogy, it might be expected that here also the liver holds the key to the situation.

The conditions in which creatin is excreted may be divided into two groups.

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* We are endeavouring to find out whether there is any relation between the creatin excretion of a pregnant woman and the development of the fœtus.

† These latter two conditions I shall consider elsewhere.

(1) Where the creatinin excreted is normal, as in pregnancy, the puerperal period, and childhood.

(2) Where the creatinin excreted is markedly subnormal, as in starvation and cancer of the liver.

In the first cases the muscle apparently retains its normal nutrition, and in the second cases there is rapid wasting. In fact, it seems likely that creatinin excretion is both an indication and a measure of the transport of material from the liver to the muscles. When this transport breaks down completely, as in carbohydrate deficiency, or when nutrient material is shunted off to supply a neoplasm in the liver, or a growing fœtus, or a developing mammary gland, then creatin is excreted. It is, at least, safe to say that most of the recent evidence of the part played by creatin and chemically allied substances which go to form the group known as "extractives" tends to show that they are concerned with the direction of nutritional transport and tissue synthesis, and that they are neither the source nor an indication of katabolic energy changes, as it has been so long supposed. As for the early period of lactation considered in this paper, the evidence leads one to believe that, in a normal suckling woman, whereas the creatinin excreted indicates the condition of nutrition of her muscles, the creatin excreted is a measure of nutriment conveyed to her mammary glands.

7. *Summary.*

In addition to confirming previous workers as to the mode of excretion of nitrogen by women after childbirth, the following new facts have been brought forward:—

1. The excretion of creatin by women, *post partum*, does not depend upon the involution of the uterus. It was shown that a woman delivered by Cæsarian section, and whose uterus was removed at the time of operation, excreted more creatin than another Cæsarian section case whose uterus was left intact.

2. Rabbits do not excrete creatin at this period. The explanation is not obvious. Eating the placenta does not explain the difference, for a cow, after having eaten its placenta, excreted large quantities of creatin.

3. A metabolic study of parturient women on creatin-free diets indicates that the creatin excretion at this time has some relation to mammary gland activity.

(*a*) There is a gradual increase in the $\frac{\text{creatin}}{\text{creatinin}}$ ratio in the first few days after delivery, corresponding with the increased mammary gland activity and the development of milk from colostrum.

(*b*) The increase in weight of healthy children breast fed under similar normal conditions is roughly proportional to the amount of the creatin in the mother's urine. In other words, the creatin excreted in the urine seems to have some relation to the nutriment given by the mother to her child.

(*c*) If owing to a toxæmic condition the activity of the breasts is delayed after childbirth, the creatin excretion is also delayed, and both develop synchronously in such cases.

(*d*) The early suppression of mammary gland activity by the development of fever and mammary gland abscesses is accompanied by the suppression of the creatin excretion. On the other hand, purgation and bandaging do not diminish the creatin excretion of the puerperium. Nor do they suppress mammary gland activity in the same way as illness does, for the breasts remain hard and knotty, and milk can usually be squeezed out for a considerable time after such treatment.

4. Feeding with casein does not affect the creatin excretion of a parturient woman.

5. The *post-partum* excretion of creatin is dissimilar from that accompanying acidosis and lack of carbohydrates. Lactose and glucose, added to the diet, do not appear to affect the creatin excretion.

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Colour Adaptation.

By F. W. Edridge-Green, M.D., F.R.C.S., Beit Memorial Research Fellow.

(Communicated by Prof. E. H. Starling, F.R.S. Received October 4, 1912,—
Read January 23, 1913.)

(From the Institute of Physiology, University College, London.)

As in dark adaptation there is a considerable effect which takes place immediately on entering a dark room, but which increases with the length of stay and the degree of darkness, so is there a considerable effect produced when a person enters a room illuminated by an artificial light, having previously been in daylight. This effect, which may be designated colour adaptation, increases with the time during which the eyes are subjected to the adapting light. I have estimated the effect of colour adaptation in four ways.

I. A dark room being illuminated by light of a certain wave-length, one eye is subjected to light of this wave-length whilst the other is closed, and is therefore in a state of more or less dark adaptation. The dark room communicates with another dark room by a door in which a hole is pierced to allow the passage of the eyepiece of my spectrometer.* A certain region of the spectrum is isolated by my spectrometer; and, after the stated period, this is examined first by the eye which has been exposed to the light, and then with the eye which has been covered up. The same spectral region is also observed after both eyes have been subjected to the adapting light.

II. The second method consists of wearing a pair of spectacles glazed with coloured glass, and noticing the changes which appear in coloured objects viewed through these glasses for a longer or shorter period. No light is allowed to enter the eye, except through the coloured glasses. As the composition of the light which passes through the glasses is known, those changes which are due to the absorption of light by the coloured glass can be separated from those which cannot be accounted for in this way. Definite spectral regions are examined first immediately after putting on the glasses, and then again after a longer period.

III. The third method is to note the changes which appear in coloured objects in a room illuminated by light of known composition, which cannot be explained by the character of the light.

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* 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 458; 'Hunterian Lectures on Colour Vision and Colour Blindness,' p. 73, Kegan, Paul & Co.

IV. The fourth method is comparing the appearance of colours in a photometer, one colour being illuminated by daylight, and the other by artificial or coloured light. The objects are then examined first by daylight, and then by the artificial light which has been used. The difference between the results obtained in this way and those of the photometer represents the effects of colour adaptation. The same colours are also examined in the photometer, both sides being illuminated first by daylight and then by artificial light.

When a spectrum is examined after the eyes have been exposed for 20 minutes in a room illuminated only by sodium light, the yellow appears to have disappeared from the spectrum. The red and green appear to meet without any intermediate colours, and the red, orange and green have lost any yellow character which they previously possessed. There is no increase in the blue or violet, and the red and green are not diminished. If, before exposing the eyes to the sodium light, a small portion of terminal red be selected, this is found to be just as visible after the exposure as before.

The same condition is found with artificial light in which the yellow rays predominate. Yellow is discriminated with difficulty from white by electric light (" Osram " incandescent). It is often impossible to detect a yellow stain on a white cloth by this electric light which is very obvious and marked by daylight.

When blue-green spectacles are first put on all white objects appear a vivid blue-green. This blue-green gradually fades until, in about 10 minutes' time, a piece of white paper or white cloth appears absolutely white, without a trace of blue-green. In fact, though I know that blue-green light is falling upon the eyes, I can see no trace of this colour. This shows conclusively how very little the conscious judgment contributes to these results, apart from the perception of relative difference. If the sky be white, misty, and overcast, this will appear only faintly coloured blue-green; if it be much brighter, or a naked filament of an electric light be looked at, these are seen as blue-green. Black objects appear black throughout. I have never been able to find the faintest tinge of red in a black object. When the spectacles are removed white objects appear a decided rose pink, and a perceptible interval elapses before objects regain their normal colour.

I found that I could read all Stilling's pseudo-isochromatic tables for testing for colour-blindness with the blue-green spectacles on. An examination of the spectrum immediately after putting on the blue-green spectacles showed that there was no red to be seen, there was a small amount of orange, and the yellow, green, blue, and violet were visible. After wearing the glasses for about 10 minutes until white appeared white, and then again examining the spectrum, there was no marked change in the orange or any other part, with the exception of the green, which looked paler and more yellow. I picked out the yellow of the spectrum by means of the shutters of the spectrometer at exactly the same wave-lengths with and without the blue-green spectacles, and with shorter and longer periods of colour adaptation. The sodium flame appears less red through the blue-green glasses, and there is no change to red after there has been colour adaptation. This shows conclusively that yellow is a simple sensation and not compounded of red and green sensations. If it were a compound sensation it should appear red after colour adaptation to green. The results are in accordance with those of colour fatigue.* The experiments on colour adaptation with the sodium light and the subsequent disappearance of yellow from the spectrum show that the yellow sensation is stimulated by the green, orange, and red rays as well as by the yellow. This is in accordance with the facts of colour mixing, and explains why red and green light make a yellow when mixed.

An examination of definite regions in the green isolated in my spectrometer shows that the region corresponding to the dominant wave-length of the glasses is most affected; the regions on the blue side and the yellow side appear bluer and yellower respectively.

The following coloured cards were used for comparison in the photometer:—

Colour by daylight.	Colour by electric light (Osram incandescent).	Colour by daylight.	Colour by electric light (Osram incandescent).
1. Yellow	Pale orange	12. Chocolate brown	Terra-cotta brown
2. Orange	Orange	13. Blue	Saturated ultramarine blue
3. Slate	Grey		
4. Blue	Blue	14. Brown	Chocolate brown
5. Yellow-green	Yellow-green	15. Dark slate	Dark grey
6. Green	Green	16. Rose red	Red
7. Brown	Light brown	17. Rose	Rose
8. Dark green	Greenish-black	18. Orange	Orange
9. Olive green	Dark green	19. Black	Black
10. Yellow	Yellow	20. Brown	Brown
11. Orange-yellow	Orange-yellow		

It will be noticed that there is very little difference in the appearance of the colours by daylight and by electric light. This is due to colour adaptation. If, however, two cards of the same colour be placed in a simple photometer which I have had constructed for the purpose, and one side be illuminated by daylight and the other side by an Osram electric light the difference is very striking. The eye which examines the colours in the instrument must have been previously in a state of daylight adaptation. It will now be found that 13 blue illuminated by electric light almost exactly matches 12 brown illuminated by daylight.

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* 'Roy. Soc. Proc.,' 1912, B, vol. 85, p. 434.

The following shows the changes in the appearance of the colours of the previously mentioned cards when two exactly similar cards are illuminated in the photometer on the one side by daylight and on the other by Osram electric light. The eye used was daylight-adapted:—

Illuminated by daylight.	Illuminated by electric light (Osram incandescent).	Illuminated by daylight.	Illuminated by electric light (Osram incandescent).
1. Greenish-yellow	Orange	11. Greenish-yellow	Orange
2. Brown	Orange	12. Chocolate	Orange
3. Slate	Brown	13. Blue	Purplish-black
4. Blue	Grey	14. Brown	Orange
5. Green	Yellow	15. Slate	Brown
6. Green	Greenish-yellow	16. Rose red	Scarlet
7. Brown	Orange	17. Rose	Orange
8. Green	Yellow-green	18. Brown	Yellowish-orange
9. Pure green	Dark yellow	19. Blue-grey	Yellow-brown
10. Greenish-yellow	Orange	20. Brown	Orange

It will be seen that colour adaptation greatly assists the correct discrimination of colours.

The same cards were now examined in exactly the same physical conditions, that is to say, two exactly similar cards were placed in the photometer, and one side was illuminated by daylight and the other by electric light. The eye used for viewing the cards was adapted to electric light by viewing white paper illuminated by electric light (Osram incandescent) for from 5 to 10 seconds. The cards used were the same as before. The following results were obtained:—

Illuminated by daylight.	Illuminated by electric light (Osram incandescent).	Illuminated by daylight.	Illuminated by electric light (Osram incandescent).
1. Green	Orange-yellow	11. Greenish-yellow	Orange-yellow
2. Buff	Orange	12. Purple-brown	Orange
3. Blue	Grey	13. Bright blue	Dark blue
4. Bright blue	Blue-grey	14. Grey	Brown
5. Green	Yellow-green	15. Slate	Grey
6. Blue-green	Yellow-green	16. Rose	Red
7. Grey	Pale orange	17. Purple	Orange
8. Blue	Black	18. Purple-brown	Orange
9. Green	Yellow-green	19. Blue-black	Black
10. Yellow-green	Orange-yellow	20. Grey	Light brown

When a match had been made to the daylight-adapted eye of chocolate brown 12 illuminated by daylight, and blue 13 illuminated by electric light, and this was viewed with an eye adapted to electric light, the two no longer matched, the blue now appeared blue and the brown pale purple.

No colour is seen by colour adaptation unless the corresponding physical stimuli are present in the light reaching the eye. On remaining in a room illuminated by light through red glass windows, green will become increasingly noticeable, but only when a certain amount of green light is transmitted by the red glass. If, however, a red glass be used which is impervious to green, not a trace of green will be seen in green or black objects. A simple yellow still appears yellow after adaptation to electric light, but a compound yellow, which appears pure yellow by daylight, and which is compounded of red and green, appears greenish-yellow after adaptation to electric light.

Colour adaptation appears to produce its effect by subtraction, and not by the addition of any new colour sensation which is not previously present. The ultramarine blue, which, when illuminated by electric light, matches a chocolate brown illuminated by daylight, appears blue after colour adaptation to electric light through the subtraction of the yellow element of the light reflected from the card. A blue sky appears much bluer when viewed from a room illuminated by electric light than it does when seen from an unlighted street, because, when viewed in the latter position, the eyes are adapted for the light of the sky, and, when viewed from the room, any yellow element is subtracted.

Summary.

1. In colour adaptation, the retino-cerebral apparatus appears to become less and less sensitive to the colour corresponding to the dominant wave-length, and to set up a new system of differentiation.

2. When light of a composition differing from that of daylight is employed to illuminate objects, an immediate and unconscious estimation of the colours of these objects is made in relation to this light, the light employed being considered as white light.

3. No colour is seen of which the physical basis is not present in the light employed.

4. When spectral regions are examined with a colour-adapted eye, that of the dominant wave-length appears colourless, whilst those immediately on either side of it appear to be shifted higher and lower in the scale respectively.

5. There is immediate colour adaptation, as well as colour adaptation after a longer stimulation with the adapting light.

6. Colours which correspond to the dominant wave-length of an artificial light are with difficulty discriminated from white by this light.

7. Colour adaptation may bring two colours below the threshold of

discrimination, so that the two appear exactly alike, although by another kind of light a difference is plainly visible.

8. Colour adaptation increases the perception of relative difference for colours other than the dominant.

9. The conscious judgment has very little effect in colour adaptation.

10. Colour adaptation greatly helps in the correct discrimination of colours and masks the effects of the very great physical differences which are found in different kinds of illumination.

11. Spectral yellow, after colour adaptation to green, still appears yellow, and not red.

12. Colour adaptation appears to produce its effects by subtraction of the dominant colour sensation, and not by directly increasing the complementary. Spectral blue does not appear brighter after colour adaptation to yellow.

The Transmission of Environmental Effects from Parent to Offspring in Simocephalus vetulus.

By W. E. Agar, Glasgow University.

(Communicated by Prof. J. Graham Kerr, F.R.S. Received October 15, 1912,— Read January 23, 1913.)

(Abstract.)

In a common daphnid, *Simocephalus vetulus,* the effects of environment (in its widest sense) on one generation may persist on generations removed from that environment, the phenomenon being clearly a case of "parallel induction" (Detto). Three main experiments were carried out, in addition to a number of experiments on related problems in the biology of the animal.

Experiment A.—The character dealt with was the ratio between the total length of the new-born individual and the width between the ventral edges of the valves of the carapace. When the animals are fed with certain protophyte cultures, the valves become rolled back in a curious way, so that in transverse section the animal is bell-shaped instead of oval, and the body appendages, instead of being nearly concealed by the carapace, are fully exposed. The distance between the edges of the valves is enormously increased, and thus the ratio length/intervalvular width correspondingly decreased.

Experiment B.—The character dealt with was the length of the new-born

animal, influenced by temperature. The length is least when the temperature is highest.

Experiment C.—Dealing with the same character as Experiment B, influenced by certain food cultures.

In the case of A and B it was found that the characters in question were acquired ontogenetically. That is to say, normal specimens were placed in the protophyte culture or high temperature soon after birth, and at maturity showed respectively reflexed valves or smaller size, compared with controls.

Animals thus rendered abnormal were removed from the stimulating environment into normal control conditions with their eggs ripe for laying. From these eggs, which only underwent their ovarian growth under the abnormal conditions, developed broods (F_1) showing their parent's abnormality very strongly marked. In later broods from the same parents still living in control conditions, the abnormality appeared in rapidly diminishing degree. The next generation (F_2) showed a very slight persistence of the abnormality, but F_3 a very pronounced reaction in the opposite direction. In all cases the effects were estimated by comparison with contemporary controls living under similar conditions.

Over 3000 specimens were measured in Experiments A and B and all were members of the same pure line, all descended by parthenogenesis from the same original female. In C fewer specimens were used, and those were not known to belong to the same pure line.

From the general results of the experiments it is concluded that in these cases the environment produces its visible effects on the soma by altering the metabolic products included in the protoplasm. These products influence the development of the soma, causing reflexion of the carapace, smallness of size, etc. Being also present in the germ plasm, they are passed passively by the gamete into the developing soma of the next generation, and influence it in the same way. Gradually the protoplasm produces antibodies to these toxin-like inclusions, and the reaction seen in F_3 is brought about.

On Negative After-Images with Pure Spectral Colours.

By George J. Burch, M.A., D.Sc. Oxon., F.R.S.

(Received October 17, 1912,—Read January 16, 1913.)

In a paper " On Negative After-Images and Successive Contrast with Pure Spectral Colours," by Mr. A. W. Porter, F.R.S., and Dr. F. W. Edridge-Green,* the authors describe certain experiments, which they consider impossible of explanation on either the Hering or the Young-Helmholtz theory of colour vision.

In justice to Thomas Young, it is only fair to point out a discrepancy between the title of the paper and the experimental conditions therein described, viz.: " The method adopted was as follows: In a dark room, *in which, however, there was a certain amount of stray light,* a horizontal spectrum, as pure as possible, was projected on a screen. A portion of the retina of one eye was then fatigued by rigidly gazing at a portion of another spectrum, isolated in the Edridge-Green colour-perception spectrometer. . . . After the fatiguing light had been viewed for about 20 seconds, the eye was turned to the screen, so that the after-image formed a band running right across the spectrum on the screen and occupying its centre."

The italics are mine. It is impossible too strongly to emphasise the fact that a spectrum projected in a room, " in which there is a certain amount of stray light," cannot be regarded as consisting of pure spectral colours.

The phenomena recorded can all be explained when the stray light is taken into account, and they agree perfectly with Young's theory. Moreover, they are familiar in laboratory practice. Thus, in paragraph 1, the effect of red light on the blue and violet, rendering these darker and bluer along the line of the after-image, is easily understood if we regard these colours as contaminated with white, the red element of which is removed by the fatigue.

In paragraphs 6, 7, 8, and 9, the fatiguing ranges were orange-yellow, yellow-green, blue-green, and blue as far as λ 475, and the after-images are said to have been purple, evidently by admixture with the violet of the stray light. But in paragraph 10, with fatiguing light λ 445–λ455, the after-image was yellow-green—clearly because the violet of the stray light was cut out by fatigue.

In paragraphs 16 to 20, experiments of a more complex character are described, all, however, capable of explanation in accordance with Thomas Young's theory, if the stray light is taken into account. This part of the paper ends with the words: "No matter what portion of the spectrum was selected, the after-image, where it crossed the spectral band, was seen as a grey square." That alone is sufficient to demonstrate the presence of stray light.

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* 'Roy. Soc. Proc.,' 1912, B, vol. 85, p. 434.

Under the head of Conclusions, the authors state that "the negative image is much darker, more difficult to produce, and more evanescent in the absence of all external light as when black velvet and the hands are placed over the eyes. It is obvious, therefore, that external light has an influence on negative after-images." I have used almost these identical words in lecturing on this subject any time these 15 years. But I have quoted them from the papers of Robert Waring Darwin, which are printed in the 'Philosophical Transactions' for 1786, and were undoubtedly made use of by Young in formulating his theory.

It is difficult to understand how anyone can expect to find acceptance for his bare statement, that "it is impossible to explain these facts on the Young-Helmholtz theory of colour vision."

I have described in my paper "On the Relation of Artificial Colour-Blindness to Successive Contrast"* various methods of observing the phenomena of successive contrast with really pure spectral colours, using stimuli no stronger than those employed by Mr. Porter and Dr. Edridge-Green. My results are different from theirs, and are in all respects quite in accordance with the theory expounded by Thomas Young.

It is a matter of everyday demonstration in the laboratory that, using moderate stimuli, with persons of normal colour sensation, yellow does change to green after fatigue to red, and to red after fatigue to green. And I am bound also to note that persons whose green sensation is weak fail to see this change in the colour of yellow after fatigue.

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* 'Roy. Soc. Proc.,' 1900, B, vol. 66, p. 206.

*Contributions to the Histo-Chemistry of Nerve: On the Nature of Walerian Degeneration.**

By Henry O. Feiss and W. Cramer.

(Communicated by Prof. E. A. Schäfer, F.R.S. Received October 22, 1912,—Read January 23, 1913.)

(From the Physiology Department, University of Edinburgh.)

[Plate 4.]

Part I.—Introductory.

When the continuity of a medullated nerve fibre is broken the peripheral segment undergoes a series of morphological changes which are grouped together under the term "Wallerian degeneration." These changes affect all the constituent parts of the fibre, namely, the axis-cylinder, the myelin sheath and the sheath of Schwann. In the early stages they consist of fragmentation of the axis-cylinder, breaking-up of the myelin sheath, and multiplication of the nuclei of the sheath of Schwann together with an increase of protoplasm around them. Although these changes have been studied by numerous observers, and although there is a general agreement with regard to their morphological appearances, different interpretations have been put upon them. And it is not difficult to understand why that should be so.

The process of degeneration is intimately related to the process of regeneration, and the differences which exist in the interpretation of the latter process are reflected to some extent in the interpretations of the phenomena of degeneration.

The degenerative changes in the axis-cylinder and medullary sheath are explained by some authors as being due to the separation of the protoplasm of the nerve fibre from the nerve cell, its nutritive centre, by others as the result of traumatism, while according to Ranvier (1) they are produced by the mechanical effect of the proliferating neurilemmal cells. The proliferation of these cells again has received different explanations. According to Ströbe (2), for instance, the neurilemmal cells are connective tissue cells, and their proliferation is of the nature of the connective tissue reaction, which takes place in the process of repair following the lesion of the specific parenchyma of any organ. According to other observers these cells are of nervous origin

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* The expenses of this research were defrayed by a grant from the Moray Fund of the University of Edinburgh.

and they play an all important part in the process of nerve regeneration. According to this latter view (5) the phenomena of Wallerian degeneration "do not deserve the term phenomena of degeneration. They are not phenomena of degeneration or death but of reorganisation or life."

We have indicated here only some of the views which are held. But this brief survey indicates sufficiently well that the various explanations of the processes of Wallerian degeneration cover a field so wide as to include their interpretation as phenomena of death on the one hand and of exaggerated phenomena of life on the other.

Although the question which thus arises is one of considerable importance, and although it can be tested experimentally, the evidence on this point is neither ample nor convincing. The importance which Ranvier attached to the proliferation of the neurilemmal cells in the process of Wallerian degeneration led him to study the changes which take place in a nerve after the death of the organism. He found (1) that the nerve of a dead animal which was allowed to lie about "somewhere in a corner of the laboratory" for several days did not show in the myelin sheath anything similar to the changes of Wallerian degeneration.

The subject does not appear to have received any attention by subsequent workers until it was taken up again by Mönckeberg and Bethe (3). These authors have apparently not been aware of Ranvier's experiments, and the observations which they record do not quite agree with those of Ranvier. They found, 24 hours after the death of an animal, a certain amount of disintegration in the axis-cylinder and the sheaths of the nerves, and these changes were more advanced when the body was kept at 37° C. But these changes were not more pronounced two and three days after death than they were after the first 24 hours. Nerves removed from a living animal and kept in a moist chamber for one or two days did not show the characteristic changes of the Wallerian degeneration in the axis-cylinder and the myelin sheath. Mönckeberg and Bethe concluded from their experiments that the process of Wallerian degeneration is dependent on the life of the fibre, and, further, that it takes place only when the nerve is lying in a medium of living tissue. The degenerative changes which do occur during the first 24 hours after death are attributed to a survival of the tissues, which is stated to be prolonged by keeping the dead animal at body temperature.

Reference must be made also to the observations of Merzbacher (4), because they have an important bearing on the problem before us. From observations on nerve degeneration in hibernating animals, and on frogs kept at different temperatures, he concluded that in the living animal the occurrence of Wallerian degeneration is dependent upon the temperature.

If the body temperature is below a certain minimum, the processes of degeneration are arrested, or, at any rate, greatly retarded.

These observations invalidate to a certain extent the conclusions of Ranvier and of Mönckeberg and Bethe. They might explain why the results of Ranvier's experiments above referred to were negative, and why in the experiments of Mönckeberg and Bethe the degenerative changes in the nerves of dead animals were more advanced when the body was kept at 37° than when the temperature was allowed to fall to room temperature.

Merzbacher also records some observations on the occurrence of Wallerian degeneration after transplantation. He found that nerves, after auto-transplantation (transplantation into another part of the same animal), and after homo-transplantation (transplantation into another animal of the same species), exhibited the typical changes of Wallerian degeneration, but failed to show them after hetero-transplantation (transplantation into an animal of a different species). These observations, as the author himself states, are only of a preliminary nature, and that accounts probably for the omission to state what changes, if any, take place in a nerve after hetero-transplantation. Moreover, in a repetition of these experiments, Maccabruni (5) has shown and illustrated by figures that hetero-transplanted nerves undergo Wallerian degeneration in the same way as auto-transplanted or homo-transplanted nerves.

From the observations of Mönckeberg and Bethe, and of Merzbacher, far-reaching conclusions have been drawn by van Gehuchten (6), which can best be stated in his own words: "There is no degeneration, and there cannot be any degeneration, except where there is life; degeneration is not only a sign of life, but it is even the most striking manifestation which we can have of an actual hyperactivity of normal cell life. Nerve degeneration is a reaction of the organism; it is an actual defence against the disturbances which a given traumatism has induced in the normal functioning of its nerve fibres."

These considerations lead van Gehuchten to demand a modification of the first part of Waller's law, which says that the peripheral segment of a cut nerve undergoes degeneration. According to van Gehuchten, this law holds good only if the significance of the word degeneration is altered, and if we understand by it "a process of life, a process of reorganisation, which by itself tends to a reconstitution of the nerve fibre."

It will be seen that all the authors who have discussed this question are agreed that the process of Wallerian degeneration is dependent upon the life of the nerve fibre. But this conclusion does not necessarily follow from their observations. In almost all the experiments, two different conditions—the life of the animal and the life of the nerve fibre—were either both operative or both excluded. It is quite conceivable, however, that the conditions which are preliminary to the occurrence of Wallerian degeneration can be established in a living animal but not in a dead animal, and that, when these conditions are established, the degenerative changes in the nerve fibre itself might proceed irrespective of the life of the fibre.

Such a possibility is mentioned here in order to indicate that the life of the animal and the life of the nerve fibre are two factors, whose influence on the process of Wallerian degeneration may have to be considered separately.

In order to obtain conclusive evidence to what extent the processes of Wallerian degeneration are dependent upon the life of the nerve-fibre, it seemed desirable to carry out observations on the behaviour of nerves removed from the body and subjected to various conditions.

Part II.—EXPERIMENTAL.

The nerves studied in these experiments were the sciatic or popliteal nerves of adult cats. For stains osmic acid was used for the myelin sheaths and hæmatoxylin for the nuclei. The osmic acid preparations were made by placing the nerves in a 1-per-cent. solution of osmic acid for 24 hours and then washing them for 24 hours. A small piece of the nerve was then removed for teasing, and the remainder was usually embedded in paraffin.

For studying the Marchi reaction, the specimens were placed in Müller's solution for one week, and then in a mixture of two parts of Müller's and one part of osmic acid (1 per cent.) for three or four days, these steps being carried out at 40° C.

All the experiments on living cats were done under complete chloroform anæsthesia, and with careful aseptic precautions if the animals were to be kept alive after the operation.

The composition of the Ringer's solution used was as follows:—Sodium chloride, 0·9 grm.; potassium chloride, 0·1 grm.; calcium phosphate solution (saturated), ad 100 c.c.

Group I.—*Excised Nerves Kept in Ringer's Solution at* 37° *for Various Periods.*

The right sciatic nerve of an adult cat was removed under chloroform, using very careful aseptic precautions. The nerve was divided into five pieces, each about $\frac{1}{4}$ inch long. Each piece was placed in a sterile Petri dish containing sterile Ringer's solution, and all the dishes placed in an incubator at 37° C. The pieces were removed at the end of one, two, three, four, and six days respectively, and fixed in 1-per-cent. osmic acid as described above.

Microscopical Findings.—The one- and two-day preparations showed few changes, although in the latter some fibres were beginning to break up. The three- and four-day preparations were distinctly changed. In many of the fibres the myelin sheath was broken up and beaded, showed vacuoles and irregular clumps of granular detritus. After six days the changes were very marked (see Plate 4, fig. 1).

These changes strongly suggested the classical signs of Wallerian degeneration as seen in nerves of living animals, following interruption of continuity.

The experiment was repeated in another series, and the findings were very much the same. Nerves were also kept in Ringer's solution for longer periods, up to 16 days, in order to see whether there would be a Marchi reaction. In no case was a positive Marchi reaction obtained.

Group II.—*Comparison of Excised Nerves Kept in Ringer's Solution at 37° C., with Nerves Degenerated in the Living for Equal Lengths of Time.*

Cat α.—Under complete chloroform anæsthesia and with careful aseptic precautions the right external popliteal nerve was freed, and a piece ½ inch long excised and placed in a Petri dish with sterile Ringer's solution. The wound was now closed. The Petri dish containing the excised piece of nerve was placed in the incubator at 37° C. At the end of one day the cat was killed and a piece of the peripheral stump of the divided nerve removed and placed in 1-per-cent. osmic acid. The specimen in Ringer's solution was treated likewise, and both specimens run through for teasing and embedding.

By this means one could compare a specimen of a nerve degenerated *in vivo* with another specimen of the same nerve kept *in vitro* for the same length of time.

Cats β, γ, and δ.—Repetition of the above, except that the lengths of time between the excision and the removal of the peripheral stump ends were two, three, and four days respectively.

Microscopical Findings.—The specimens kept in Ringer's solution presented the same sort of appearance as those described under Group I. Very similar to these changes were the changes to be observed in the specimens of the same nerves which were actually degenerated in the living.

There was, however, this difference. Although the degenerated fibres were more or less broken up, and the myelin collected in elongated or round masses, the stain was usually clear and well diffused. In the specimens kept in Ringer's solution there was more detritus, and the myelin in many of the fibres showed a lack of clearness in its staining reaction. This appearance of the myelin might be described as "flaky," while that of the fibres degenerated *in vivo* might be referred to as "laked."

Paraffin sections, made from the specimens kept in Ringer's solution, were stained with hæmatoxylin, and showed the nuclei broken up and staining badly.

The next group of experiments was carried out in order to determine the effect of temperature on the changes which nerve fibres undergo *in vitro*.

Group III.—*Comparison of Excised Nerves kept in Ringer's Solution at* 37° *and at* 5° *respectively.*

External popliteal nerves were removed aseptically. Each nerve was divided into two pieces and placed in Ringer's solution in Petri dishes. One set of dishes was placed in the incubator, the other set placed in the ice box.

At the end of four and six days the nerves were removed and placed in 1-per-cent. osmic acid.

Microscopical Findings.—The nerves kept at 5° showed the characteristic changes in the myelin sheath, but these changes were not as pronounced as those seen in nerves kept at 37° for the same lengths of time.

The next group of experiments was devised in order to test whether contact with an aqueous solution was a contributory factor in bringing about these changes in the myelin sheaths. Attempts to keep nerves in the incubator in a moist chamber for several days, so that they were hanging freely in the chamber and did not come into direct contact with the solution, did not give very satisfactory results, because the nerves dried partially. The object of this group of experiments was attained, however, by immersing the nerves in liquid paraffin.

Group IV.—*Comparison of Excised Nerves kept at* 37° *in Ringer's Solution with Excised Nerves kept in Liquid Paraffin.*

External popliteal nerve removed from an adult cat under sterile precautions, and divided into two pieces. One piece was placed in Ringer's solution. The second was placed in liquid paraffin. Both specimens kept at 37°. Pieces removed at end of six days and fixed in 1-per-cent. osmic acid.

Microscopical Findings.—The specimen kept in Ringer's solution showed the characteristic signs described above under Group I. The specimen kept in liquid paraffin consisted for the most part of apparently normal fibres.

Knowing the effects of Ringer's solution on nerve fibres, the question arose whether the fluid constituents of the blood would have the same effect. This experiment was carried out in the ice box, in order to exclude the effect of bacterial contamination.

Group V.—*Comparison of Excised Nerve kept in Ice Box for Six Days in Ringer's Solution with Excised Nerve kept in Blood Serum under Similar Conditions.*

Cat chloroformed and external popliteal nerve removed under sterile precautions. The nerve was divided into two pieces and temporarily laid in a sterile dish. The carotid artery of cat opened under sterile precautions, and some blood allowed to flow into a Petri dish. The blood was allowed to clot and the dish then tilted so as to cause the serum to flow to one side. The one piece of nerve was laid in this serum, and the other was laid in a Petri dish containing sterile Ringer's solution. Both dishes were placed in the ice box. Pieces were removed at the end of six days and fixed in 1-per-cent. osmic acid.

Microscopical Findings.—Both specimens showed the changes described under Group I. The changes were about equal in amount.

One of the essential differences between the conditions of a nerve degenerating *in vivo* and a nerve kept *in vitro* is the presence in the former of a circulatory mechanism, which not only supplies the fibre with nutriment, but in this case may also have an effect in removing products of degeneration. In order to estimate the part played by this factor it was necessary to study the changes in a nerve kept *in vivo* in the absence of any circulation. This condition was obtained by tying a nerve in two places as described in the following group.

Group VI.—*Nerves tied in Two Places in Living Cats. Comparison of Preparations made from the Segment between Ligatures with Preparations taken from Segment below Lower Ligature.*

Under complete chloroform anæsthesia external popliteal nerve freed and tied very tightly with catgut ligatures in two places ¾ inch apart.

Cat killed five days later. External popliteal nerve freed again. The portion of the nerve between the ligatures shrunken to about one-half its normal size. The portion below the lower ligature was somewhat swollen. Pieces from between the ligatures and from below the lower ligature fixed in 1-per-cent. osmic acid.

Microscopical Findings.—Preparations made from the segment below the lower ligature showed characteristic signs of a nerve degenerated for five days, while preparations from the segment between the ligatures showed the peculiar flaky appearance of the myelin that was seen in nerves kept in Ringer's solution. The contrast in the appearance of the two preparations was very marked, although the amount of breaking up of the myelin was about equal in the two segments. (See Plate 4, figs. 2 and 3.)

The difference obtained between the two segments in this experiment suggested the possibility that the interference with the circulation might have an effect on the onset of Marchi's reaction. In five cases the external popliteal nerves were tied between the ligatures. The nerves were removed after 8, 11, 12, 13, and 14 days respectively and the Marchi reaction in the segment between the ties compared with that in the segment below the ties. It need hardly be pointed out that by this method the circulation was not cut off for the whole length of time during which degeneration took place.

The 8- and 11-day specimens showed no Marchi reaction. The 12- and 13-day specimens showed beginning Marchi reactions. In neither case was there any appreciable difference between the segments compared. The 14-day specimen showed a more positive Marchi reaction. Here again there was no appreciable difference between the segments compared.

SUMMARY.

Cats' nerves removed from the body and kept at body temperature in Ringer's solution or in blood serum exhibit certain changes in the myelin sheath as studied in osmic acid preparations, which resemble the early changes exhibited by nerves degenerated for about equal lengths of time in the living. These changes are slowed but not inhibited by lower temperatures. In nerves kept in liquid paraffin, the changes are not seen to occur to any great extent.

There is one difference in the appearance of nerves degenerated *in vivo* from that of nerves kept *in vitro:* the broken down myelin stains less clearly in the latter condition, and thus has a flaky appearance. This same flaky staining was noted in the living when the circulation of a nerve was cut off locally.

Nerves kept in Ringer's solution *in vitro* showed no Marchi reaction and no signs of nuclear activity.

CONCLUSIONS.

In discussing the nature of the changes comprised under the term Wallerian degeneration, we must consider separately the proliferation of the neurilemmal nuclei and the fragmentation of the myelin sheath. That the former is a manifestation of life goes without saying. The fragmentation of

Feiss and Cramer. *Roy. Soc. Proc., B. vol. 86, Plate 4.*

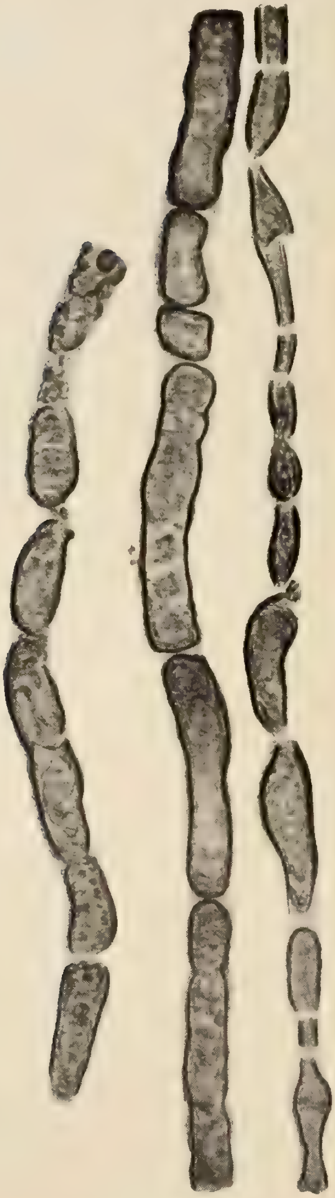

FIG. 1.

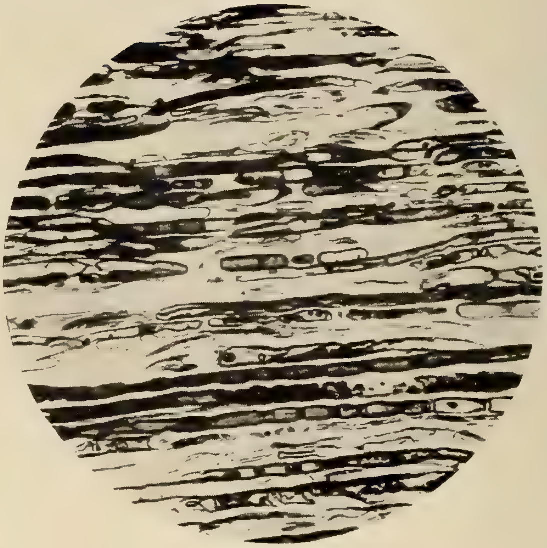

FIG. 2.

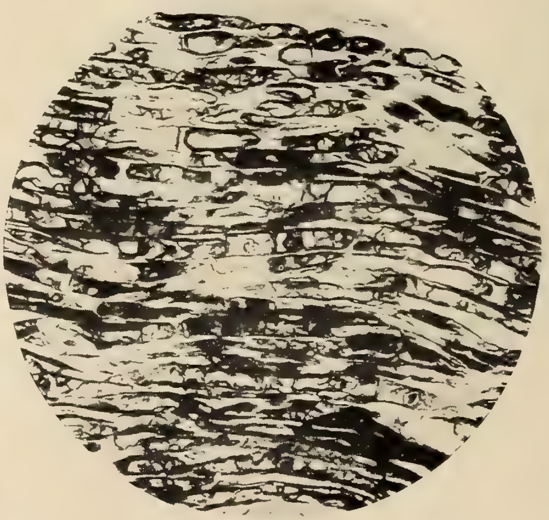

FIG. 3.

the myelin sheath, however, is not dependent upon life, since it occurs in nerves removed from the body and kept under certain conditions, in which the nuclei show no signs of proliferation.

It follows also that the changes in the myelin sheath are not dependent upon the proliferation of the neurilemmal nuclei, as has been held by some authors.

The fact that the fragmentation of the myelin sheaths in nerves kept *in vitro* is not markedly inhibited by cold indicates that the changes in the myelin sheaths are not essentially due to processes of a fermentative or autolytic nature.

On the other hand contact with an aqueous solution appears to be of essential importance in bringing about the fragmentation of the myelin sheaths in nerves kept *in vitro*. A process of imbibition appears, therefore, to be a contributory factor in bringing about the changes in the myelin sheath characteristic of Wallerian degeneration.

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DESCRIPTION OF PLATE 4.

Fig. 1. Cat's nerve kept in Ringer's solution at 37° for six days. Osmic acid. Teased preparation. × 500 diam.

Fig. 2. Microphotograph of cat's nerve degenerated *in vivo*, five days after having been tied in two places. Segment below lower tie. Osmic acid. Cut in paraffin. × 200 diam.

Fig. 3. Same nerve as fig. 2. Segment between ties.

Factors Affecting the Measurement of Absorption Bands.

By H. Hartridge, M.A., Fellow of King's College, Cambridge.

(Communicated by Prof. J. N. Langley, F.R.S. Received November 1, 1912,—
Read January 16, 1913.)

(From the Physiological Laboratory, Cambridge.)

In a previous paper a method was described* by which the percentage saturation of hæmoglobin with carbon monoxide can be estimated. This was done by measuring with a special spectroscope the position of the absorption bands of a solution of blood, since it was found that a definite relationship exists between the percentage saturation with carbon monoxide and the wave-length of the bands. The principle used in the instrument was one first discovered by Zöllner in 1870 and called by him the reversion spectroscope. Two adjacent reversed spectra were obtained by passing beams through a slit suitably placed in relation to a reflecting prism and a replica diffraction grating, optical means being employed for shifting one of the spectra laterally, so that corresponding points in the spectra might be adjusted into line. Since first describing the method I have been able to investigate more thoroughly its accuracy both in my own hands and also in those of other observers. Two different classes of phenomena will receive attention, both of which tend to introduce complications in the use of the method when absolute values for the percentage saturation with carbon monoxide are required. These are:—

(*a*) Variations in wave-length determinations made from time to time by same observer on different samples of blood (personal variation).

(*b*) Variations in wave-length determination by different observers on same sample of blood (individual variation).

Description of Personal Variation.

As a result of wave-length measurements of the bands of the O_2 and CO compounds of hæmoglobin, I found that those previously published differed considerably from my own; further, in these there was found from time to time a small but quite definite divergence. Both these points are shown in the table below.

It will be seen from the table that by my measurements the change in wave-length of the α-band, when O_2 is replaced by CO in the hæmoglobin compound, is 53·3 Ångström units;† a value which is lower than that given by the other observers quoted. This may have been due to dissociation of

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* 'Journ. Physiol.,' 1912, vol. 44, p. 1.

† Ångström unit = 10^{-8} cm.

Table I.—Wave-length Measurements on Hæmoglobin Bands.

Observer.	α-band.			β-band.			Date.
	O_2.	CO.	Difference.	O_2.	CO.	Difference.	
H. H. ……	5764	5710 ·5		5403 ·5	5363		
	5764	5713		5405	5357 ·5		
	5765 ·5	5713 ·5		5400	5361		
	5766	5709		5402	5362 ·5		
	5764 ·5	5712 ·5		5401 ·5	5363		
	5763 ·5	5710		5402	5364		
	5763 ·5	5711		5402	5361		
	5765 ·5	5710		5402 ·5	5360		
	5763	5709 ·5		5404 ·5	5361 ·5		
	5764	5712		5405	5364		
	5764 ·5	5711 ·1	53 ·3	5402 ·8	5361 ·5	41 ·3	1912
Gamgee……	5790	5720	70	5438	5380	58	1880
Formanch…	5781	5710	71	5417	5375	42	1901
Dilling ……	5785	5715	70	5420	5378	42	1909
Heubner* …	5769			5402			1912

* 'Bio-chem. Ztsch.,' 1912, vol. 38, p. 345.

the COHb by light, since it was considered inadvisable to employ any screening fluid, in case this influenced the normal positions of the bands. Ammonium sulphide has the property of rendering the CO compound much more stable to light; measurements done in the presence of this body gave a value as high as 59 A.U. Variations were, however, still found in the absolute values, in one case the wave-length varied as much as 5·4 A.U. from the typical position. Alterations in the scale of the instrument were in every case avoided by making the measurements from two standard lines, sodium supplying that for the α-bands, the thallium line 5351 A.U. for the β-bands. These differences in wave-length must be due to one of two causes, either to actual differences in the wave-length of the various blood solutions measured, or to retinal changes brought about by alterations in the intensity of the incident light, or the previous stimuli received by the eye. Before dealing with the explanation of these phenomena I will proceed with the description of the second, somewhat larger, individual variation.

Description of Individual Variation.

Measurements of wave-length carried out by several observers on the same blood solution showed that considerable divergence of opinion could exist as to the apparent positions of the absorption bands. In this case also the first question to require consideration was the cause of the divergence, but the effect this might have on the accuracy of the carbon monoxide estimations had also to be investigated. The differences that may exist between the readings of two observers may be seen from fig. 1.

On the left are plotted the observations and calibration curve obtained from them for the observer G. W. On the right is drawn a portion of my own calibration curve taken from the previous paper.* On comparing corresponding points on the two curves it will be seen that the bands appear everywhere displaced by about 20 scale divisions (10 A.U.). The initial observations of G. W. are given in the table below and are shown plotted as black dots.

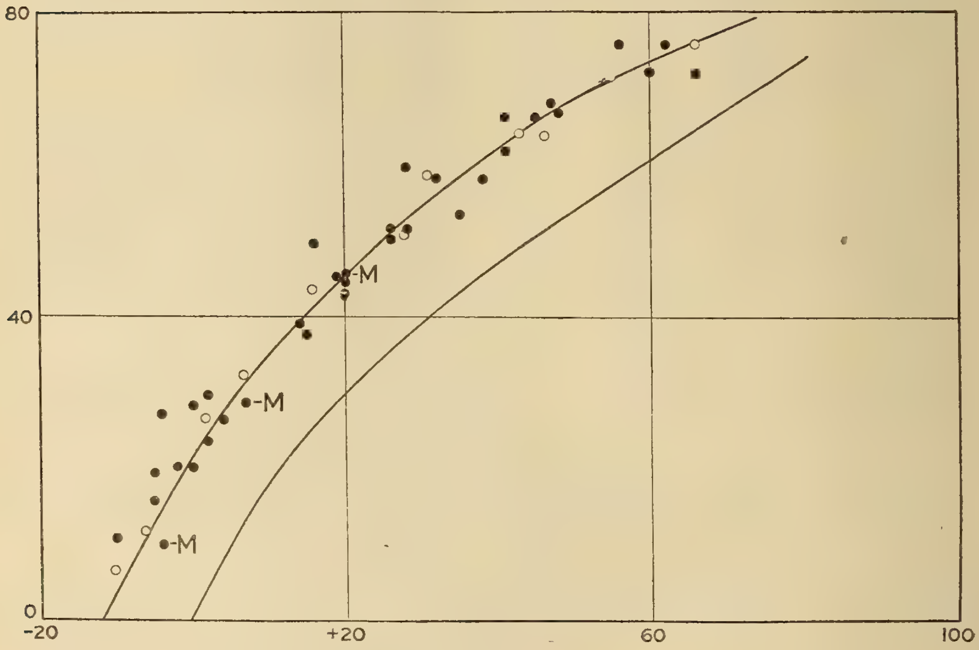

Fig. 1.

●, initial observations ; M, mean of several readings ; ○, final observations.

But to ascertain how far subsequent readings could be relied on to give the actual percentage saturation of a sample of blood, a further series of isolated readings were made. These are shown plotted in fig. 1 as white dots, and are given in the table side by side with the actual percentage saturation present; it will be seen that the latter readings have an average error of 1·1 per cent. These figures show that although a considerable

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* 'Journ. Physiol.,' 1912, vol. 44, p. 23.

Table II.—Observations for Obtaining and Checking Calibration Curve.
Observer G. W.

Initial observations by which calibration curve was obtained.

CO.	Reading.	CO.	Reading.
per cent.		per cent.	
75·8	56	10·1	M− 4·5
20·5	− 2	45·8	19
27·5	− 4	16	− 5
75·8	62	72	60
62	41	39·4	14
45	+20	29	M 7
20·5	0	68·2	47
46	M 20	28·5	0
19·7	− 5	11	−10
30	2	52	26
60	28	58·6	38 and 32
66·5	45	43·5	20
50·6	26	66·5	41
26·8	4	52	28
38	15	23·9	2
72	66	67	48
50	16	53·8	35

M = mean of 4 or 5 observations.

Final observations used to check accuracy of calibration curve.

Actual CO.	Reading of spectroscope.	CO calculated from curve.	Difference.
per cent.		per cent.	per cent.
50·6	28	53	2·4
32·4	+ 7	31	−1·4
75·8	66	76	+0·2
6·7	−10	− 3	−3·7
43·5	16	41	−2·5
63·6	46	66	+2·4
58·6	31	55·4	−3·2
26·8	2	24·3	−2·5
12	− 6	10·5	−1·5

Average error −1·1.

difference may exist in the curves of different observers, yet the final results obtained by them need not vary greatly from the true value; the variations depending largely on the degree of practice of the observer.

Experimental Demonstration of the Factors that Control the Position of Boundaries and Mean Wave-lengths of Absorption Bands.

Theoretical considerations showed that the factors of importance are :—

(1) The number of molecules of pigment encountered by light in transmission (*i.e.* concentration × thickness).

(2) The initial intensity of incident light.

(3) The activity of the retinal response.

(1) The effects of alterations in concentration or thickness of the solution under examination are well known, having been first investigated by Rollett.* These observations have been repeated with the new method, the results being given in the table below and shown graphically in fig. 2.

The widths of the bands increase with an increase in concentration or thickness of the solution till a point is reached when fusion of the neighbouring bands takes place. The mean wave-lengths of the α- and β-bands, on the other hand, do not appear to be affected by changes in concentration, and this would point to the bands being symmetrical in form. Mention has

Table III.—Effect of Change in Concentration on Mean Wave-length and Boundaries of O_2Hb Bands.

Hb.	A.	B.	C.	D.	E.	F.	G.	H.	I.	J.
per cent.										
0·1	7260		5764				5403			4210
0·2	7260	5800	5765	5738	5600	5485	5401	5345		4420
0·3		5810	5765	5733	5600	5487	5403	5340		4590
0·4		5829	5765	5716	5602	5490	5400	5310		4690
0·5		5837	5762	5703	5604	5505	5397	5300		4750
0·6		5843	5765	5685	5607	5526	5396	5290		4830
0·7		5856	5759	5678	5608	5540	5395	5265		4880
0·8	7210	5860		5678	5612	5546		5250		4950
0·9	7160	5865			5610			5230	5130	5000
1·0	7130	5863						5226	5126	5010
1·1		5864						5210	5123	5010
1·2		5876						5180	5123	5030
1·3		5880							5125	
1·4		5886								
2·0	7000	5900								

A, the apparent commencement of spectra in the infra-red.
B, the yellow edge; D, the green edge; C, the mean wave-length of the α-bands.
E, the centre of unabsorbed area between α- and β-bands.
F, green edge; H, blue edge; and G, mean wave-length of β-band.
I, centre of unabsorbed area between β- and γ-band.
J, green edge of γ-band.

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* Schäfer, 'Text-Book of Physiol.,' vol. 1, p. 233.

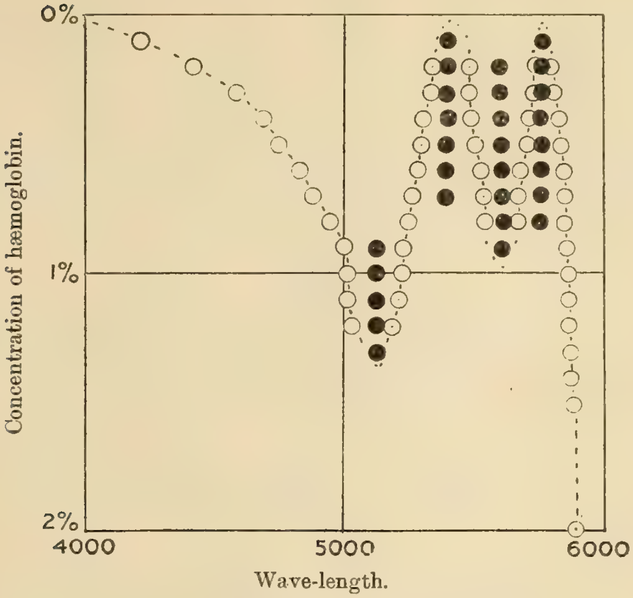

FIG. 2.—Sheep's Blood, 1 cm. thick. ○, apparent edges of bands; ●, mean wave-length.

been made elsewhere* of the possession of symmetry by these bands, this is of considerable theoretical importance, and is to be more fully investigated at a future date.

(2) The effect of alteration in intensity of the incident light on the boundaries of the bands of O_2Hb is shown in the following table:—

Table IV.—Effect of Changing Intensity of Incident Light on the Bands.

Intensity.	A.	B.	C.	D.	E.	F.	G.
8	5812	5764	5698	5598	5492	5398	5316
4	5823	5766	5698	5594	5501	5403	5314
2	5833	5764	5692	5595	5500	5404	5302
1	5841	5763	5688	5583	5511	5402	
$\frac{1}{2}$	5840	5765	5684	5591		5396	
$\frac{1}{4}$	5852	5765		5588		5402	
$\frac{1}{8}$	5856	5766		5596		5391	
$\frac{1}{16}$	5868	5765		5592		5393	

A. Yellow edge } of α-band.
B. Centre } of α-band.
C. Green edge } of α-band.
D. Centre of unabsorbed area.
E. Green edge } of β-band.
F. Centre } of β-band.
G. Blue edge } of β-band.

It will be noted that a decrease in the intensity of the light tends to make the bands broader, whereas an increase makes them narrower. In this case also evidence is afforded of the symmetry of the bands. Limiting the change of intensity to that part of the spectrum occupied by one side of a band still causes the same change as those described above, an increase in intensity making that side of the band narrower, a decrease making it broader, the change being always such as will cause the band, as a whole, to appear shifted away from the side of greater intensity (see Table V).

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* 'Journ. Physiol.,' 1912, vol. 44, p. 8.

Table V.—Effect of Changing Intensity of Incident Light on One Side of the Bands.

Normal band.	Increased intensity.		Decreased intensity.	
	Green.	Yellow.	Green.	Yellow.
5765	5767 5768	5760 5762	5759 5760 5760	5773 5771 5774

Gelatin films, stained with methyl orange and malachite green, were used to reduce the intensity of the incident light in the green and orange regions of the spectrum respectively. The increased intensity was obtained by supplementing the light used above by a Nernst lamp, which could be fitted with light filters that transmitted either the orange or green parts of the spectrum.

(3) It is well known that by decreasing the intensity of the light incident on the retina, a point is ultimately reached where no sensation of light can be obtained. This point is called the "visual threshold." The amount by which the light has to be decreased to reach the threshold depends on the activity of retinal response. This not only varies with the wave-length of the light but also with the stimuli received by the retina previous to the threshold measurement. Thus, when the previous stimulus has been intense, a light of considerably greater intensity than the threshold may cause no sensation.

Moreover, a stimulus of a certain wave-length not only affects the threshold for that wave-length, but also to a less extent that of wave-lengths on either side; in cases where the previous stimuli have been very intense and prolonged every part of the spectrum becomes affected.

The position of the visual threshold at different points of the spectrum is one of the factors which determine the appearance of absorption bands; this is well shown in the case of oxyhæmoglobin in Table VI.

Previous stimulation by a bright light causes the threshold to rise and makes the bands become wider; a fall in the threshold conversely makes

Table VI.—Effect on Band Widths of Altering Value of Threshold.

Strength of solution	0·4 per cent. Hb.	0·6 per cent. Hb.
Normal eye	5829–5712	5826–5700
After keeping in dark	5811–5726	5852–5691
After stimulation by a bright light	5845–5694	5859–5680

The measurements are of the α-band.

them narrower. Further, if the bands be measured after alteration in the threshold on one side of the band only, then a movement of the mean wave-length of the band will be found to have occurred; this being in every case towards the side on which the threshold is highest.

Table VII.—Showing Change in Wave-length of Bands produced by Alterations in Retinal Adaptation.

CO.	Normal position of α-band.	A.	B.
per cent.			
24	5757·5	−1·5	+3
39·4	5750	−1·1	+2
10·1	5762	−1·5	+2·1
53·8	5739	−1	+2·5
68·2	5728·5	−1	+3·6

The readings in Column A were taken after the eye had been blinded for green rays by a powerful light; in Column B, the light used was orange.

Lastly, there is another important factor, viz., contrast, the full effects of which are to receive future consideration. By comparing in several cases the visual appearance of absorption bands with their spectrophotometer curves, the following observations have been made in nearly every case:—

(1) A certain density gradient is necessary for an absorption band to be recognised.

(2) Any gradient that exceeds this tends to be increased in slope by contrast.

(3) Light and dark areas connected by such gradients increase in width.

(4) Areas of high intensity become brighter, those of low intensity darker.

(5) Small density changes within such areas tend to become obliterated.

(6) A line breaking the continuity of a gradient causes a considerable increase in the differences of density on the two sides.

The method of observation and data will appear at a future date.

Fig. 3 shows these six effects, it also demonstrates how contrast impairs the accuracy of measurement, either of the mean wave-length or of the apparent boundaries of bands by the cross-wire and micrometer. Where such a method is alone available, the use of a broken line is indicated.

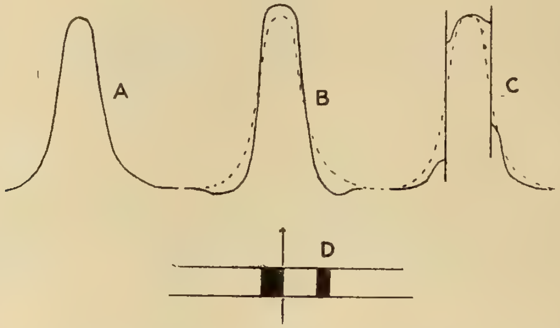

Fig. 3.—To show the Effects of Contrast on the Appearance of Absorption Bands.

A, normal density curve; B, curve altered by contrast; C, effect of breaking continuity of density gradient by the line of the micrometer; D, suggested use of "broken" line in the micrometer.

A brief theoretical consideration may now be given of the manner in which threshold and intensity of incident light control the appearance of absorption bands. Any desired value may be given to the density at any point of the spectrum, by causing the incident light, on transmission, to encounter the correct number of pigment molecules. Any part of a spectrum may therefore be made to fall below the value of the threshold of vision, and, when such is the case, these portions must give to the eye the appearance of uniform black areas having no visible luminosity. This explains why, by starting with a dilute solution of such a pigment as hæmoglobin, and gradually increasing either its concentration or thickness, a point is reached at which a narrow black area appears in the centre of the α-band, and, further, why this area increases in width on either side as the concentration or thickness is further increased, until fusion of neighbouring areas ultimately takes place. Similar changes may clearly be brought about by altering either the initial intensity of the incident light or the threshold. In fig. 4 these changes are shown diagrammatically.

Such considerations explain the existence of certain conditions upon which the accuracy of the estimation of CO in blood is found to depend. As much of the bands as possible should be below the threshold, without the conditions being such as to impair the luminosity of the area separating the α and β components. These optimum conditions are always aimed at when making CO estimations; their existence is of great practical importance, and is well shown in fig. 5.

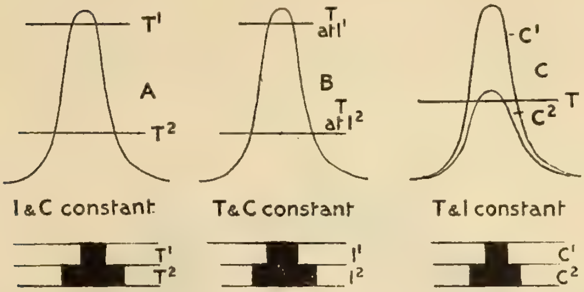

FIG. 4.—To show the Effect of Changes—A, in the visual threshold ; B, in the initial intensity of the light ; C, in concentration—on the Width of Absorption Bands.

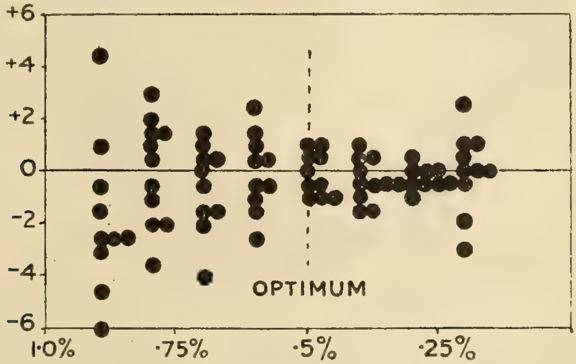

FIG. 5.—To show the Existence of an Optimum Concentration at which Best Measurements are to be obtained.

The alterations have been considered above as taking place uniformly throughout the spectrum. It remains to consider the case when alteration on one side only of a band is involved.

It has been shown above that a rise in the threshold or a fall in the intensity of the incident light both have the same effect, namely, to increase the width of the bands. Where one side only of a band is affected, it is clear that a change in the mean wave-length of the band will be involved, since one side of the band increases in width, the other remaining stationary. This will cause the band as a whole to appear shifted towards that side on which the intensity reaches its lowest, or the threshold its highest value. This is shown diagrammatically in fig. 6.

It will be remembered, when describing the personal and individual variations in the wave-length of the bands of hæmoglobin, that even under the same conditions differences are still found to occur; these are to be explained as being due to alteration in the threshold. For observers are agreed that the threshold value for light of different wave-lengths is a factor

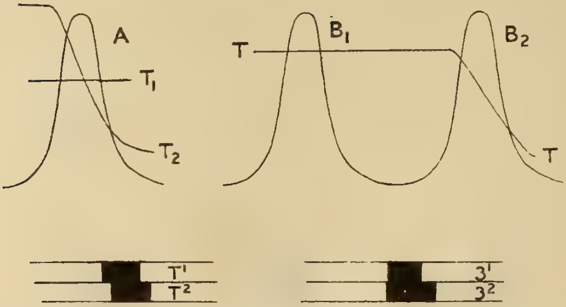

Fig. 6.—To show the Effect of Changes in the Threshold or Intensity of Incident Light, taking place on one side of a band only, on the mean wave-length of the band. At A the value of the threshold on one side has been changed; at B′ and B the normal intensity of the incident light has been altered, moving with it the threshold.

which not only varies greatly with the individual, but is also modified by the quality and time of the stimuli the retina has previously experienced.* In the case of the observer G. W. and myself, a difference of 10 A.U. was found in the wave-length of the bands; this would point to an abnormality in the threshold values of the green or yellow regions of the spectrum for one of us. To test whether this was accompanied by any change in the normal intensity of sensation, tests were carried out by Rayleigh's method,† a yellow being matched with a combination of red and green; we were unable, however, to demonstrate any difference in our sensation of these colours.

The variations demonstrated above in the visual measurements of wave-length of absorption bands are also to be found when a photographic process has been employed; for colour, sensitive plates have a threshold at different wave-lengths which depends on the dyes used to sensitise the emulsion. Further, the actinic value of the light used to illuminate the slit of the spectrograph should be as far as possible adjusted to be of uniform intensity throughout the spectral region under examination. For this purpose the cadmium arc and similar light sources which yield a number of fine lines of very varying intensity are unsuitable. When a suitable source has been chosen the true density gradient may be ascertained by determining experimentally the threshold values at nine or ten evenly distributed positions in the desired spectral region. It thus becomes possible to obtain for the infra-red and ultra-violet portions of the spectrum density curves that are the equivalent of spectro-photometric measurements in the visible spectrum.

It is interesting to observe that the factors of contrast, etc., here described may in their relation to the phenomena of absorption bands be conveniently investigated without the use of spectral apparatus; all the effects being obtained by means of a rotating disc calculated from the spectrophotometer curve of any chosen band. Such a disc is shown in fig. 7.

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* 'Physiology of Special Senses,' Greenwood, p. 104.

† Rayleigh, 'Collected Papers,' vol. 1, p. 543.

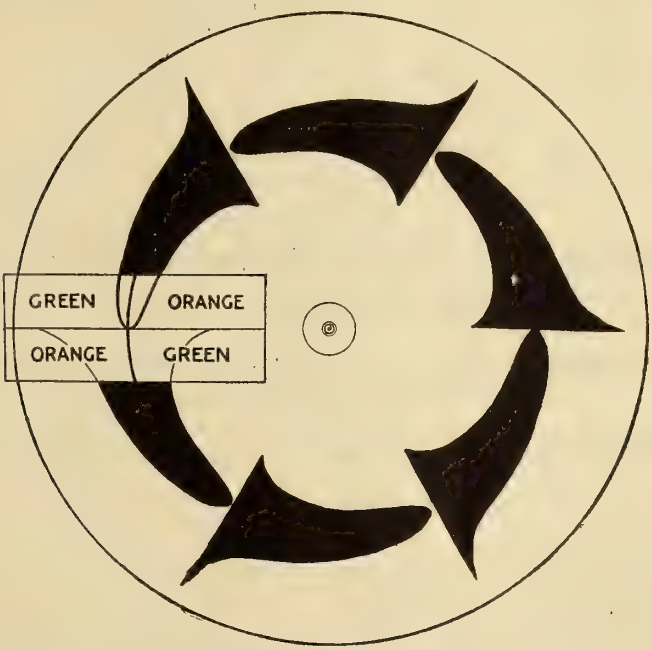

Fig. 7.—Diagram of Colour Disc used for imitating Absorption Bands and Effects of Changes in Intensity and Threshold.

Summary.

(1) When absolute values of the CO saturation of hæmoglobin are required the spectroscopic method is complicated by the fact that each observer must obtain a calibration curve for himself, and this should also be checked from time to time against the blood from the particular source under examination.

(2) These individual differences are due to the particular threshold values at different wave-lengths.

(3) The differences greatly detract from the value of accurate wave-length measurements of absorption bands, both visual and photographic.

(4) They do not, however, prevent accurate CO estimations being made, provided that care be taken to work under standard conditions.

(5) The effects are considered of variations, on one or both sides of an absorption band, in—

(*a*) The initial intensity of the light.

(*b*) Value of threshold and adaptation of retina.

(*c*) Contrast.

I wish to thank Mr. G. Winfield for the trouble he has taken in making himself familiar with the use of my spectroscopes. I am also most grateful to Prof. Gotch for the kind suggestions he made to me early in the present research as to the best line of attack; these have been of the greatest assistance.

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*The Phenomenon of "Narcosis Progression" in Mammals.**

By T. Graham Brown (Carnegie Fellow).

(Communicated by Prof. C. S. Sherrington, F.R.S. Received November 12, 1912,—Read January 23, 1913.)

(From the Physiological Laboratory of the University of Liverpool.)

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I. *Introduction.*

At present the subject of "reflex walking" is one almost entirely neglected by physiologists, yet it is of fundamental importance in the physiology of the nervous system. For this reason it is desired to lay stress upon it by means of the present paper, which is to be regarded as a preliminary account of a phenomenon which will later be described in greater detail.

The present author has already described certain movements of progression

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* The expenses of this research have been defrayed by a grant from the Carnegie Trust. The results here described were embodied in a thesis presented to the University of Edinburgh on March 31, 1912.

which occur in rabbits while subjected to the state of ether or chloroform narcosis.* These movements are of interest in that they exactly resemble the peculiar form of locomotion in that animal—simultaneous movements of the hind limbs ("hopping") and alternate movements of the fore limbs. An additional point of interest is that scratching movements may occur also in narcosis, and the phenomena of narcosis movements may slide, as it were, from the one type into the other.

In previous papers read before the Society, mention has been made of the fact that similar movements (that is, of progression) may be induced under narcosis in the guinea-pig† and in the cat.‡

The movements in the guinea-pig are of interest because they do not occur under normal conditions of ether or chloroform narcosis—at any rate, they have not been observed in a long series of experiments, although sometimes after an operation and when the animal is recovering from the narcotic it may make such movements. Under normal conditions the movements which occur in the guinea-pig under narcosis are those of scratching—the "narcosis scratch."§

In the cat scratching has not been seen by me to occur in narcosis amongst about 200–300 individuals which I have observed in this state. On the other hand, movements of progression occur with great frequence, probably in about 50–75 per cent. of cats subjected to general chemical narcosis induced by ether or chloroform or by a mixture of the two.

In the cat the progression movements are best seen in the hind limbs, where they may appear when the fore limbs exhibit no movement. They exactly resemble the movements of progression in the normal animal. Sometimes the two hind limbs move simultaneously (or nearly simultaneously) in the same direction, but more usually the narcosis progression is alternate. It may rarely be confined to one of the hind limbs.

The rhythmic phenomenon may be transient, when it appears then lasting but a few seconds. In other cases it may last for long periods of time. Records in which the movements have continued unchanged for several minutes have been obtained. In such cases the narcosis must be kept at a constant level. If the depth be increased the movements decline in extent and finally die out. If the depth of narcosis be decreased the movements cease somewhat suddenly when in full strength.

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* 'Quart. Journ. Exper. Physiol.,' 1911, vol. 4, p. 151.

† 'Roy. Soc. Proc.,' 1912, B, vol. 84, p. 555.

‡ *Ibid.*, 1912, B, vol. 85, p. 278.

§ 'Journ. Physiol.' ('Proc. Phys. Soc.'), 1909, vol. 38, p. 86; 'Quart. Journ. Exper. Physiol.,' 1910, vol. 3, p. 21; *ibid.*, 1911, vol. 4, p. 19; *ibid.*, 1911, vol. 4, p. 151.

Of the three chief joints of the hind limbs the ankle joint is that at which the movements of narcosis progression in the cat are best seen. Where the phenomenon is well marked it may appear at all three joints; intermediate grades may be characterised by movement at knee and ankle; where slight, movement only at the ankle may be observed. Even where there is no obvious movement there it is sometimes possible to feel rhythmic "beats" of tibialis anticus by palpation of its tendon of insertion in front of the ankle.

Although these movements of narcosis progression must have been observed in the cat by many previous investigators, to the best of my knowledge they have not before been minutely examined and described. They are of interest on account of the light which they throw upon the phenomenon of progression in comparison with the various other rhythmic phenomena which may be observed. Such rhythmic phenomena may be seen in the scratch reflex, in simple reflexes, in compounded reflexes, as reflex rebound, in response to central stimulation of the cut surface of the spinal cord, in the progression phenomenon which may follow a rapid division of the spinal cord, and in the narcosis movements here described. In some experiments several of these forms of rhythmic phenomena have been obtained.

II. *Methods Employed.*

In the guinea-pig the movements of the intact hind limbs have been registered upon the moving surface of the kymograph through the mediation of a pair of levers, which were connected to the hind limbs by means of threads. During the taking of a record the animal was laid upon its back, being sustained with its long axis parallel to the table upon which it rested.

When the movements of narcosis progression in the intact hind limbs of the cat were registered, the animal was placed prone upon the table. A hot-water bottle was placed under the lower part of the abdomen and had the effect of raising the pelvis. A steel bar (parallel to the table at a distance of about 5 cm. and at right angles to the threads connecting the toes to the recording levers) was placed under the ankles. The movements then registered were almost only those at the two ankle joints.

The movements at the ankles have been observed after various operative procedures. Thus they have been examined after decerebration of the animal by rapid division of the brain stem through the anterior colliculi; after rapid division of the spinal cord in the region of the lower thoracic segments; after de-afferentation of one hind limb by the division of the posterior spinal roots proper to it; after motor paralysis of groups of muscles in the two hind limbs; and, finally, in two individual muscles (*gastrocnemius* and *tibialis anticus*—antagonists at the ankle joint) after motor paralysis of all the other muscles of both hind limbs.

III. *Narcosis Progression in Guinea-Pigs.*

In the normal condition under narcosis the rhythmic movements which occur are those of the scratch. On an attempt being made to induce these after local anæsthesia of the receptive skin fields for the scratch-reflex by means of the subcutaneous injection of "novocain" (0·1–0·2 grm.), it was found that movements of progression occurred, and that scratching could not be obtained. This accidental discovery was confirmed in other cases, and it must appear that the application of this drug causes the narcosis movements of the guinea-pig to change from those of the scratch to those of progression. This result is not conditioned by the site of the application of the drug, for it is obtained after intra-peritoneal injection. In cats in which narcosis progression has not occurred, an injection of "novocain" may sometimes be followed by their appearance.

When these movements occur in the guinea-pig they are bilaterally alternate in the two hind limbs. Their amplitude may be greatly increased during the application of a peripheral stimulus. Such an augmentation, for instance, occurs if mechanical pressure be applied to the fold of skin which passes from the abdomen to the front of the thigh. In this case the amplitude of the "beats" is increased in the limb of the same side and diminished in that of the opposite side.

The usual rate of rhythm of the movements is about 2·0 beats per second in each hind limb (two complete cycles of progression per second). This has been remarkably regular. In one and the same individual variations of rate of rhythm between the extremes of 1·75 and 3·0 beats per second have, however, been observed.

In this same individual the movements of the narcosis scratch were recorded on another occasion. The rate of rhythm of the beats of the scratch varied between 7·0 and 8·0 beats per second. This rate corresponds very closely with the average rate of 7·5 beats per second obtained from a large number of different individuals.* It seems to bear the simple relationship of 4 : 1 to the rate of the movements of progression in the same individual. If this is the case it is of interest that the relationship should be 2 : 1 in the case of the rabbit† (where the progression is that of hopping), and of 4 : 1 in the guinea-pig (where the progression is that of running).

IV. *Narcosis Progression in Cats: Intact Hind Limbs, Bilateral Progression.*

The movements in the intact hind limbs, as studied at the ankle, are of flexion followed by extension—rhythmically repeated. Perhaps it would be more correct to say that the movements are of flexion followed by relaxation of flexion—because palpation of the extensors usually fails to detect any movement. There is probably a reciprocal relaxation during the flexor contraction, and a restitution of maintained tonus during flexor relaxation, but there is no evident contraction in this phase.

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* 'Quart. Journ. Exper. Physiol.,' 1910, vol. 3, p. 21.

† *Ibid.*, 1911, vol. 2, p. 151.

The movements occur in the two limbs. They are then related in such a manner that flexion at one ankle occurs during relaxation of flexion at the other; and when the second limb exhibits flexion the first relaxes.

It happens (although rarely) that sometimes the movements in the two hind limbs are nearly synchronous, in the sense of being in the same direction at the same time.

Graphic records of the movements of the two ankles during this narcosis progression demonstrate the relations of the movements in the two hind limbs, and also the variations of their rates of rhythm.

In a typical instance the curve traced by each foot is rhythmically discontinuous (fig. 1). Each remains for intervals parallel with the abscissa—corresponding with a posture of extension. Between these intervals each curve describes a sharp rise and fall (+ and − flexion). This movement may be termed a "beat"; and there is usually no pause at the top of a beat. The movement of relaxation of flexion (or of extension) immediately succeeds that of flexion contraction. Occasionally there is a slight pause at the top, and the curve then remains parallel with the abscissa in a posture of maintained flexion. The fall of a beat is usually a more rapid movement than the rise.

When the tracings of the two ankle movements are compared it is found that the beat of one falls within the pause of the other. The exact relationship depends upon the rate of rhythm of the movements.

Where the movements are slow the intervals may be of greater duration than the beats. In such a case the top of a beat in one foot corresponds in time to the mid-point of an interval in the other. The end of an interval in one foot then overlaps the beginning of one in the other. Immediately after each beat there is a short period during which both feet are in a state of maintained extension. This is then terminated by the appearance of the following beat at the other ankle.

If the rate of rhythm is faster and the duration of the pauses exactly equal to the duration of the beats then the termination of a beat at one ankle is immediately followed by the commencement of a beat at the other; the termination of that by the commencement of a beat at the first—and so on.

With still faster rates the interval between beats at either ankle becomes shortened. The apex of a beat at one ankle then falls mid-way in this pause, but the commencement of the beat occurs at a point during the relaxation phase of a beat at the other ankle. The relaxation phase of the first beat then synchronises at a certain point with the point of commencement of a beat at the second—and so on.

Increase in the rate of rhythm is then accompanied by a disappearance of

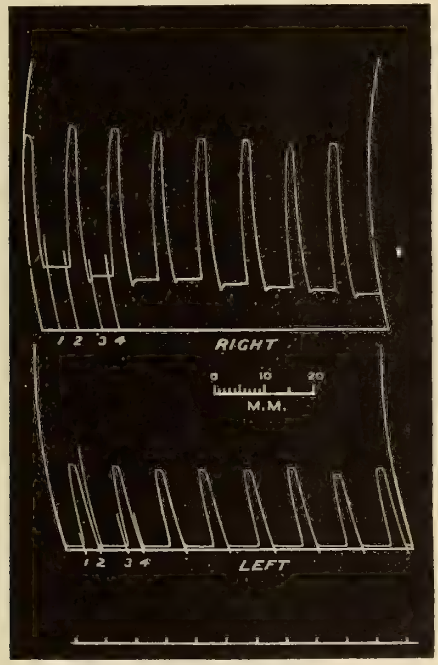

Fig. 1.—Experiment C, XXXIV, record 60; 11.4.11.—Normal cat, record of narcosis progression obtained by registration of the movements at the ankles of the intact hind limbs.

The movements are here slow. It will be observed that there are distinct pauses in flexion relaxation between the beats, and that there are slight pauses at the top of the beats. The pauses in relaxation are of greater duration than are the beats. The beats are alternate in the two hind limbs. The corresponding ordinates marked 1, 2, 3, 4 demonstrate that the commencement of a beat at the left ankle occurs after the commencement of a pause between beats at the right, and that the termination of that beat occurs before the termination of the same pause. Thus, for instance, immediately after ordinates 4 both limbs for a short time are in flexion relaxation.

In this and in all the other figures, except figs. 7 and 8, the upper tracing is that obtained from the movements of the right foot and the lower that from the left. The rise of the curve denotes flexion, and the fall extension at the ankle. Corresponding ordinates on the two tracings (usually numbered 1, 2, 3, etc.) demonstrate the time relations of different points. A millimetre scale has been drawn before the record was varnished, and is thus reduced in proportion with the rest of the record. The lowest line registers time in seconds.

the pause between the beats of either foot. At each ankle the commencement of a beat follows immediately upon the termination of the preceding beat. The apex of a beat at one ankle is then found to correspond in time with the point of transition from beat to beat at the other.

Where the movements of narcosis progression are fast it may happen that they are no longer bilaterally alternate at the two ankles, but are more or less exactly synchronous. The progression is then that of the gallop.

The rate of rhythm in different experiments has been found to vary between comparatively wide limits. Thus a rate as slow as 0·6 cycle per second has been registered. (A cycle may be measured by taking the average duration of time between successive apices of the beats at one ankle.) Rates as fast as 2·5 cycles per second have been registered under normal conditions; while under asphyxia in addition to the narcosis the rate has been found to rise as high as 3·3 beats per second—or even higher.

Even in one and the same individual the rate may vary considerably. Thus on two successive days in one individual rates of 0·6 and 2 cycles per second have been registered. Nine days later the rhythm was 1 cycle per second.

The movements are not always regular. Sometimes "grouping" may occur. The beats may then occur in pairs with shorter pauses between the elements of a pair than between successive pairs. Other variations may occur, and sometimes there may be a dropping out of the movements of a limb for short intervals of time during the registration of a long record.

V. *Narcosis Progression in Cats: Intact Hind Limbs, Unilateral Progression.*

Unilateral progression as regards the pair of hind (or of fore) limbs is of course a phenomenon often seen in the case of the mammal which uses quadrupedal progression. Three-leg progression in the dog after injury to one limb is a common sight. It occasionally happens that the phenomena of narcosis progression are entirely confined to one hind limb; while it more often happens that for short periods of time a bilateral progression in the hind limbs becomes unilateral.

In the forms of unilateral progression in which the phenomenon is entirely confined to one of the pair of hind limbs there is little to describe. The rate of the movements is almost always very fast, and corresponds to that which obtains in bilateral progression when the movements are synchronous in the two limbs (galloping).

Temporary abolition of the movements of progression in one limb of the pair may take place during long records of bilaterally alternate movements (fig. 2).

This abolition occurs suddenly. The beats in one limb fail, and there ensues a shorter or a longer pause during which the beats occur alone in the

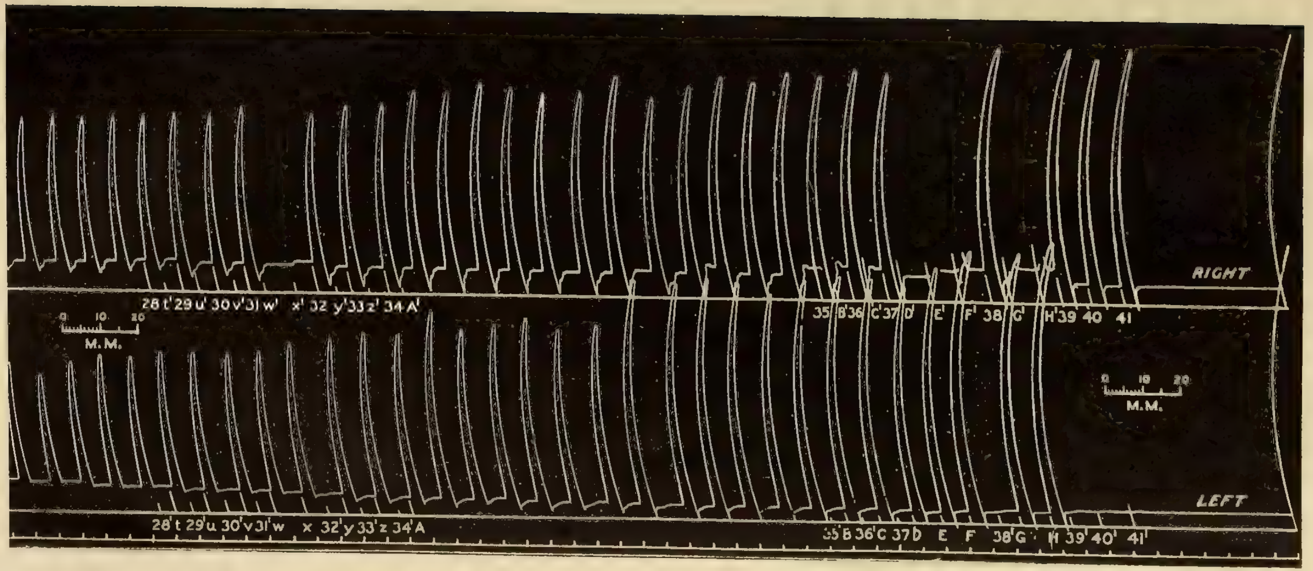

FIG. 2.—Experiment C, XXXIV, record 69; 21.4.11.—Normal cat (the same one from which fig. 1 was obtained 10 days before), record of narcosis progression. This figure gives the last part of a tracing in which the movements were recorded for a period of 100 seconds. The movements were at the commencement of 0·5 of the extent of the last ones here reproduced. As the depth of narcosis was decreased they gradually became larger until they suddenly stopped. This tracing is typical of the sudden cessation of the phenomenon under these circumstances. It will also be observed that towards the end of the tracing the beats tend for short intervals to become unilateral. When this occurs here, the beats in the limb which continues to move are quicker than before. This may be seen in the case of the right limb at ordinates 39, 40, 41; the distance between ordinates 39 and 41 (= 2 cycles) is shorter than that between ordinates 35, 37 (where both hind limbs are moving). The same phenomenon is more marked in the left hind limb at ordinates D, E, F (unilateral, = 2 cycles) as compared with ordinates B, C, D (bilateral, the two cycles immediately preceding the unilateral phenomenon).

other limb. Reappearance of the beats in the first limb then terminates this pause; but these beats are at first smaller than usual. The common extent of the beats is soon attained.

The duration of the long pause is approximately equivalent to that of so many cycles. That is, it is approximately equal in duration to a simple multiple of the duration of a single cycle when the two limbs are acting together alternately. This correspondence is, however, only approximate; for minuter examination of the records reveals the fact that the pause is in duration smaller than would be this simple multiple. The beats which continue in being in the other limb become more rapid in rate of rhythm than before. The change of rate of rhythm in such temporary unilateral progression may be slight. It may change from 0·75 to 0·9 cycle per second only. In other cases it may change from 1·5 to 2·5 cycles per second.

The beats of a limb in which the movements occur during the temporary abolition of the beats in the other are often increased in extent as well as in rate of rhythm. This increased extent may persist for a short time after the resumption of movement by the other limb. It then gradually disappears synchronously with the attainment by these beats of their normal extent.

VI. *Narcosis Progression in Cats: the Effect of some Lesions of the Nervous System.*

The movements of progression in the intact hind limbs may be recorded —as they occur at the ankles—after certain lesions of the central and peripheral nervous systems.

Of these, the first which we may mention is that of decerebration by rapid division of the brain stem through the anterior colliculi.

In one experiment the movements of narcosis progression were recorded before decerebration, and also 15 seconds after the infliction of the lesion. Before decerebration the movements were very well marked, and were alternate in the two hind limbs. After decerebration they were very much reduced in extent, the reduction being greater in one limb than in the other. The movements were synchronous in the two limbs at the beginning of the record, but later they became alternate. The rate of rhythm also changed after decerebration. Before it had been about 1·2 cycles per second. Afterwards it became about 2·6 cycles per second. The movements entirely disappeared 40 seconds after decerebration.

The movements of narcosis progression in the hind limbs may persist after local injury to the lumbar region of the spinal cord.

Thus, after the movements of normal narcosis progression have been recorded, the lumbar spinal cord may be transected at the level of the entry of the most caudal fibres of the VIth post-thoracic posterior spinal root without abolishing the narcosis progression. This lesion, if accompanied by the destruction of the lower part of the cut cord, removes from the body the centres for the extensors of the ankle joint, and leaves that for the flexors (or part of it). Yet the movements at the two ankles may still persist.

Again, in the same experiment, the remaining portion of the lumbar spinal cord has been split in the middle line of the body in the VIth, Vth, and lower part of the IVth post-thoracic segments, and the left part of the cord in these segments has been removed. Movements of narcosis progression persisted in the right hind limb at the right ankle. They were very slight, and it was almost impossible to register them, but palpation of the tendon of tibialis anticus revealed the fact that that muscle continued rhythmically to contract, and it appeared that the rate of the movements was about twice as fast as before.

The movements of progression in this experiment before the first lesion (in the normal condition) had been of good extent and of a rate of about 0·95 cycle per second. After the first lesion the extent of the beats was reduced, but their rate remained at about 1 cycle per second. After the second lesion the extent of the beats was very markedly reduced, and the rate of rhythm appeared to be about 2 cycles per second. At the time of registration the beats appeared to be of a rate of about 1 cycle per second—but possibly of about 1·3 cycles per second.

Division of the posterior spinal roots of one of the two limbs in another experiment was followed by abolition of the narcosis movements as they occurred in that hind limb. The movements in the other limb continued, but were slower than before. Only one experiment of this sort has been successful. It is not usual for the movements of narcosis progression to last as long after the commencement of narcosis as is necessary for the preparation of the spinal cord and roots before they are cut. It is, perhaps, remarkable that the movements should survive the procedure in any case. It is but fair to add that in this successful experiment the narcosis progression had at the commencement of narcosis showed a marked tendency to be unilateral in either hind limb, and especially in that the posterior roots of which were not cut.

In another case motor paralysis of one hind limb was produced by division of all its spinal roots—both motor and afferent. In this instance the movements of narcosis progression persisted in the other hind limb, but were then more slow than before.

In yet another experiment the movements of narcosis progression at one ankle survived not only the motor paralysis of all the muscles save the knee extensors of the other hind limb, but also the motor paralysis of all the muscles (save the knee extensors) acting upon its own hip and knee joints. The movements at this ankle still survived after transection of the lumbar spinal cord at the level of the lower border of the VIIth post-thoracic segment, and also after division at the lower border of the VIth segment.

Finally, I have lately divided the spinal cord in the lower thoracic region very rapidly while the narcosis progression (in deep anæsthesia) was in being. In this instance the movements were not recorded, but there appeared to be little change in them. They ceased about 30 seconds after the lesion.*

VII. *Narcosis Progression in Cats: in Individual Muscles.*

When the individual antagonists at the ankle (tibialis anticus and gastrocnemius) are prepared for the registration of their movements all the muscles of the other hind limb are put out of action by motor paralysis, while all the other muscles of the same hind limb are similarly paralysed. After this drastic procedure it is not strange that the movements of progression narcosis are difficult to obtain—especially as they soon tend to disappear in the normal cat as the narcosis is continued for any great length of time.

The movements of progression narcosis have, however, been recorded in the individual muscles, and in an experiment in which they were also recorded in the intact hind limbs.

In the intact hind limbs the movements were of good extent, regular, and of a rate of about 1·6 cycles per second. They were recorded for a period of about 90 seconds.

After preparation of the individual muscles at the ankle the movements persisted and were very well marked. The extensor—gastrocnemius—throughout exhibited no trace of movement; but the flexor—tibialis anticus—presented a record composed of well marked beats, regular in extent and rate of rhythm. These were composed of contraction phases immediately succeeded at the summit of contraction by relaxation. The termination of the phase of relaxation was succeeded by a pause in which the muscle remained in relaxation, and then contraction again appeared (fig. 7).

The rate of rhythm of these beats was about 1·5 cycles per second, so that here the motor paralysis of one hind limb and the motor paralysis of all the muscles of the other save only the two retained had no apparent effect upon the rate of rhythm of the movements.

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* *Note* (*added* January 23, 1913).—I have lately repeated this result, with graphic registration. The narcosis progression was occurring at a depth of anæsthesia so great that rapid division of the lower thoracic spinal cord evoked scarce a movement. Again, I have found narcosis progression to occur at a depth of anæsthesia at which reflex movement at the ankle could hardly be elicited. To these recent observations I would like to add a third: narcosis progression may persist through the procedure of decapitation, and thereafter may suddenly merge, in one hind limb, into the scratch-reflex.

The general appearance of this tracing was such that to inspection it might easily be mistaken for one in which the movements of the intact hind limb were recorded.

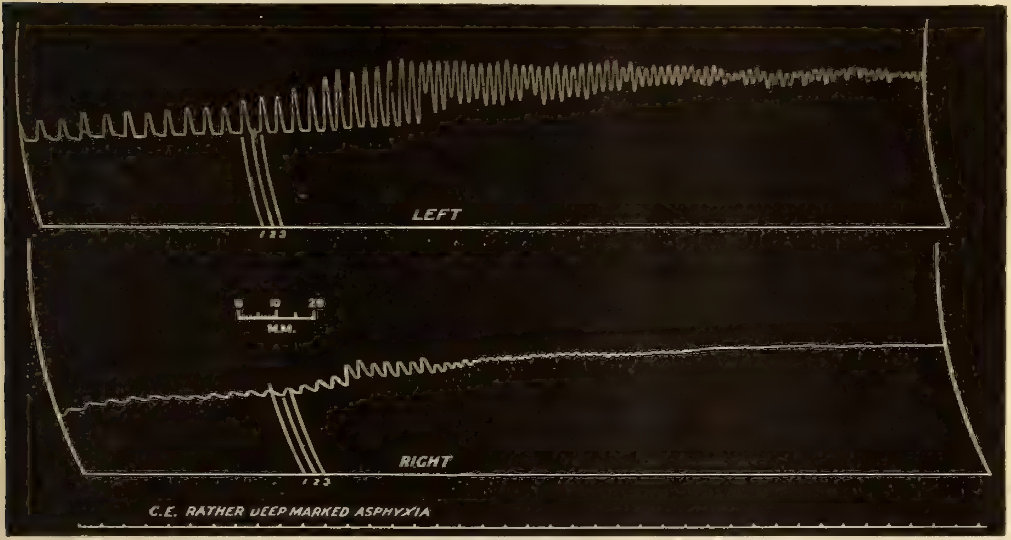

Fig. 3.—Experiment C, LXII, record 110; 19.7.11.—Normal cat, narcosis progression under the influence of chloroform-ether narcosis reinforced by the application of 0·2 grm. "novocain." Effect of asphyxia—trachea closed 1–2 seconds before ordinates 1. In the left limb the beats are seen for a time to continue to relax to the same level, but to become of increased extent and rate of rhythm. Then the relaxation becomes less complete, and as this becomes still less the beats decrease in extent, although their height does not fall. In the left limb the beats do not disappear, but in the right they disappear and a state of maintained flexion is left. In the left limb the pauses between the beats are seen to disappear. The beats in the two limbs are at first alternate (as at ordinates 1, 2, 3), but when they are of maximum extent in the left limb they are very nearly synchronous. Later they are again alternate.

VIII. *Narcosis Progression in Cats: the Effect of Asphyxia.*

In the cat under narcosis the effect of asphyxia may easily be studied by completely closing the elastic trachea (or by closing a tracheal cannula when that has been inserted). In these experiments this complete closure was applied for short periods—about 15 seconds to about 50 seconds (maximum). An asphyxia of about 30 seconds is sufficient to give the effects to be described. These have been examined both in the intact hind limbs and in the individual antagonists at the ankle. The records demonstrate many minor variations in the phenomenon. The description here is that of the typical effect.

A short period of complete asphyxia produces three kinds of effect upon movements of narcosis progression then in being. These are: Change in the rate of rhythm of the beats; change in the extent of the beats; and change in the mutual relations of the beats in the two limbs.

Change in Rate of Rhythm.—Two main phases occur between the commencement of asphyxia and the attainment of its complete effect. The first of these is a slowing of the rate of rhythm. This may be absent, or it may be so great as to cause complete cessation of the beats. It seems to be conditioned by an increase in the pauses between the beats. In extent of duration it is usually slight; a rate of rhythm before closure of the trachea of about 1·3 cycles per second may become one of about 1 cycle. This phase lasts for about 9–12 seconds, at the end of which time the rate of rhythm again increases (or the beats reappear if they have been suppressed). The phase of the increase of rate of rhythm is the second. It is never absent unless the beats at the commencement of asphyxia are already very fast. The rate of rhythm becomes progressively more fast, at first by a reduction of the pauses between beats, but later (when the pauses have disappeared) by a reduction of the duration of the beats. This phase may last for as long as 20 seconds, and the rate of rhythm may become thrice that which obtained before the application of asphyxia. Thus a rate before asphyxia of 1·3 cycles per second has been observed to change to one of 3 cycles per second 25 seconds after closure of the trachea; and one of 1 cycle has changed to 2·75. The beats themselves may shrink in duration to 0·75 of the duration before asphyxia.

Change in Extent.—Synchronously with these changes in rate of rhythm, changes in the extent of the beats may appear. This change may be measured either by the examination of the heights of the beats (that is, of the heights of the apices of maximum flexion); or by an examination of their lengths (that is, of the distances between the apices and the lowest points in the beats). If the apices of the beats in an asphyxia record be joined by an imaginary line, and if the lowest points be joined all together by another, the beats will appear as conditioned in height at any one point by the distance between the two curves there. The first curve—which is that of maximum flexion—is parallel with the abscissa at the time of the application of the asphyxia. It then sometimes slightly falls (the fall may, of course, be great—as when the beats disappear in the first phase). This fall is soon succeeded by a gradual rise of the curve, and it is most common for this gradual rise to commence at once and without preceding fall. Throughout the phase in which the rhythm slows the curve of maximum flexion gradually rises, and it continues to rise throughout the whole phenomenon until the complete

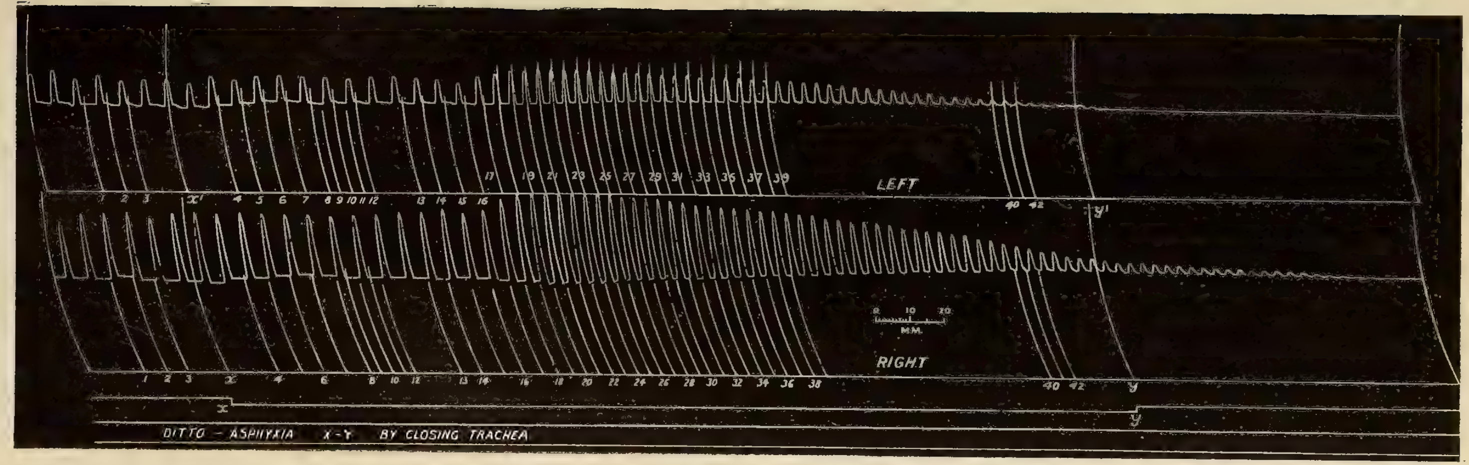

Fig. 4.—Experiment C, LXXVIII, record 133; 4.11.11.—Normal cat, narcosis progression under chloroform-ether anæsthesia. Asphyxia (by complete closure of the trachea) was commenced at x of the signal line (ordinates x, x') and stopped at y (y, y'). The other ordinates, numbered 1–42, mark on both tracings the point of commencement of the right beats. It will be noticed that here the latency of the asphyxia effect is comparatively long; the beats markedly increase in extent and rate; but the pauses do not disappear, the beats do not become bilaterally synchronous, the state of maintained flexion is badly marked, and the beats do not disappear entirely in the right hind limb.

Here the time marker failed: 1 second = 6 mm. (reduced scale) in length along the abscissa.

asphyxia effect is obtained. Towards the end of the second phase the rise becomes much less rapid than at its commencement, and the curve finally becomes again parallel to the abscissa as the maximum effect is reached. The curve of minimum flexion—that joining the lowest points in the beats—may also fall in the first phase. If it does so its fall is less than that of the curve of maximum flexion, and more usually it remains parallel with the abscissa throughout the first phase. At the end of that phase, and when the beats are again increasing in rate of rhythm, this curve commences to rise more rapidly than that of maximum flexion. This rise continues to be more rapid until the maximum asphyxia effect is attained. In fact, both curves continue to rise throughout the phenomenon. That of maximum flexion has a shorter latency and a more gradual rise than the curve of minimum flexion, which has a longer latency and a more rapid rise. In consequence of this the beats are of greatest relative extent (that is, from apex to their lowest point) at the end of the first phase when the curve of minimum flexion is just about to rise, and thenceforward they progressively diminish in extent—at the same time becoming increasingly more rapid. The maximum effect is attained when the curve of minimum flexion coincides with that of maximum flexion. Just before this is attained the beats are very small in extent and very fast. When it is attained they disappear and there is left in their place a state of maintained flexion. This maintained flexion may continue to increase for a short time after this.

Change in Bilateral Relations.—The temporal relations of the movements in the two hind limbs are of interest during this phenomenon of asphyxia. At the commencement of the condition it may be supposed that the beats in the two hind limbs are accurately alternate. In the first phase of the asphyxia phenomenon, when the beats slow in rate and increase in extent, this relationship persists. Thereafter a change makes its appearance in the relationship of the beats on the two sides of the body. The apex of the beat on one side at this point falls midway between the apices of two beats on the other. In successive beats the apex then leaves the mid-point and either advances or retires towards the first of the two apices of the other limb between which it falls (fig. 5). This process is continued until the apex of a beat of one limb actually coincides with that of a beat in the other. The movement is then that of the gallop—synchronous beats in the two hind limbs. This state often persists for the remainder of the period of asphyxia, but sometimes the beats become again less completely synchronous.

This phenomenon of progression in asphyxia, if it is induced in one of the rare cases in which the progression is already synchronous, may not interfere with that synchronism. The beats may become slightly more fast.

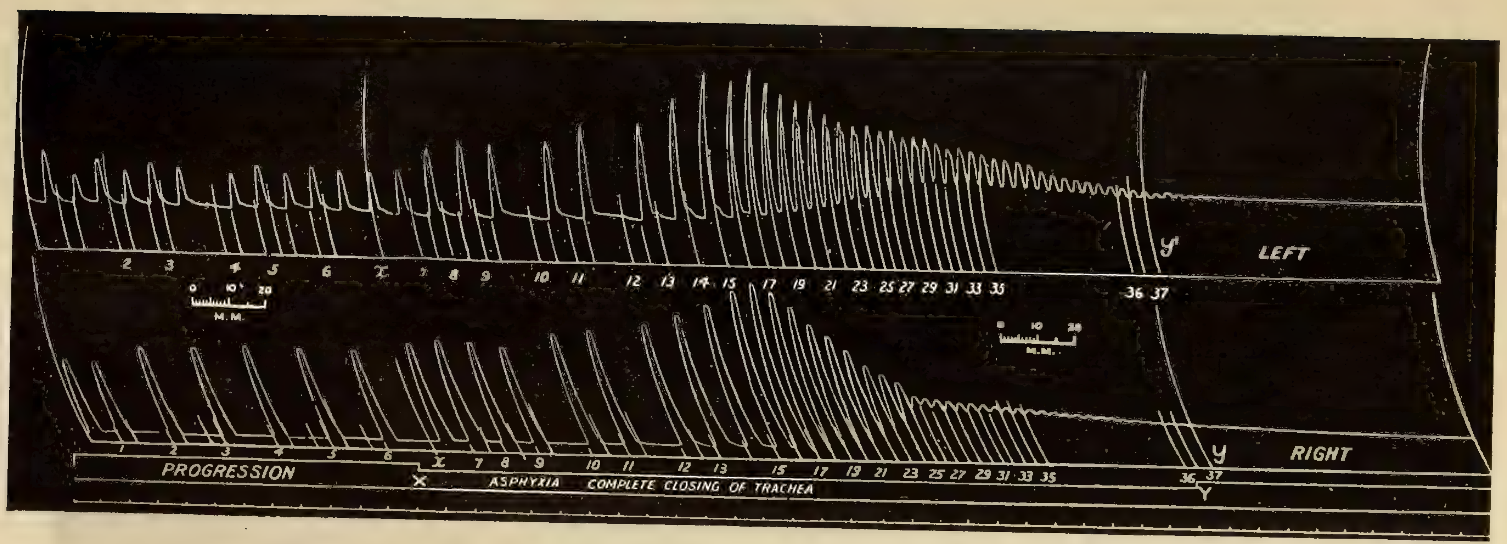

Fig. 5.—Experiment C, LXXXIV, record 140; 16.11.11.—Normal cat, narcosis progression. [The paper was torn in varnishing in a diagonal line between ordinates 6–7 below and 8–9 above: it has been carefully fitted together.] Asphyxia was applied between X–Y. A curious reduplication of the beats in the left hind limb is seen before the commencement of the asphyxia.

This record demonstrates (1) the increase in the extent of the beats in both limbs; (2) increase in their rate of rhythm; (3) disappearance of the pauses between beats; (4) late decrease in the extent of the beats, merging in (5) a state of maintained flexion (not so great or so well marked as in some cases); and (6) change from bilateral alternation of the beats in the two limbs to bilateral synchronism.

The last phenomenon is most clearly marked, and is demonstrated by the relations of the numbered ordinates, which all correspond with the commencement of beats in the *right* limb. At the time of the disappearance of pauses from between the beats of the right limb (*cir.* ordinates 19) the beats are alternate. Thereafter the relationship *gradually* changes until at about ordinates 33 the beats are synchronous in the two hind limbs, as is shown by their synchronous commencement and termination.

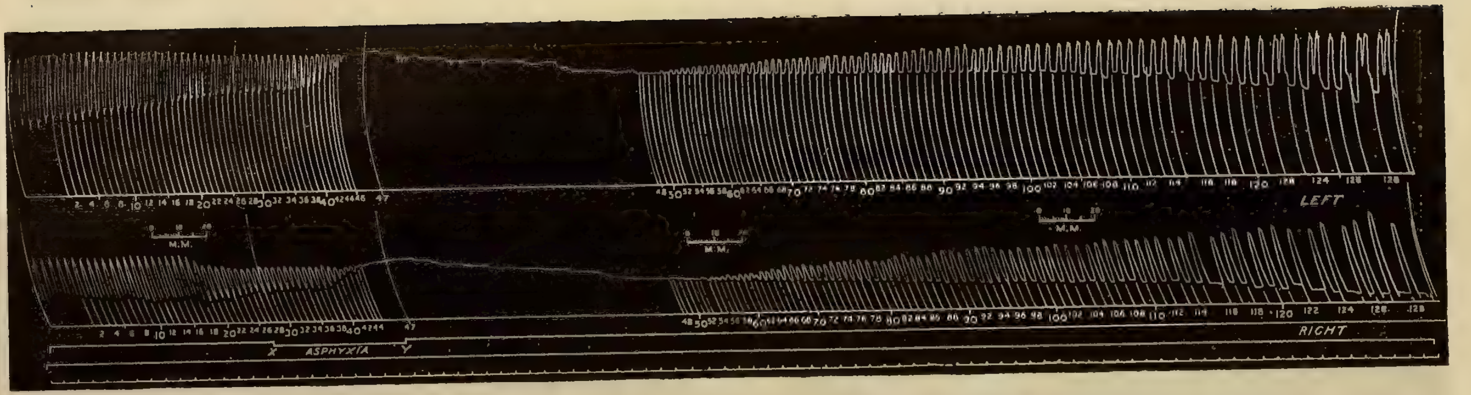

Fig. 6.—Experiment C, XCVII, record 158; 15.12.11.—Normal cat, narcosis progression. Here the movements are decreasing in extent when asphyxia is induced. The beats are rapid in rate of rhythm and nearly synchronous even before the application of the asphyxia. The state of maintained flexion is marked in both muscles, and just before the full effect is attained the beats are small and rapid. This tracing demonstrates the recovery from asphyxia. There are first short series of small and fast abortive beats. Then real recovery commences and the beats become larger and slower as the process continues. At the end of the record the beats are much slower and larger than at the beginning before asphyxia. This in part may be due to a lessening of the depth of narcosis.

It sometimes occurs that, if the movements of narcosis progression have occurred in a cat but have then ceased, the production of a state of asphyxia may induce them again. They then appear some time after the commencement of asphyxia at that point at which it might be expected that the beats would be reaching their maximum extent.

Recovery.—The asphyxia effect ends in the production of a state of maintained flexion. If the asphyxia be continued there may then be no recovery of the movements of progression. But if the asphyxia be terminated whenever the complete effect is attained—or, better still, a few seconds before it is attained—the movements of progression again make their appearance (fig. 6). Even when the asphyxia is stopped before the attainment of the complete effect (that is, when the beats are still present) the full effect is attained. The state of maintained flexion then persists for a few seconds; the beats reappear—being then of small extent and rapid rate of rhythm; become of greater extent and of slower rate; and finally again attain their normal appearance. The maintained flexion

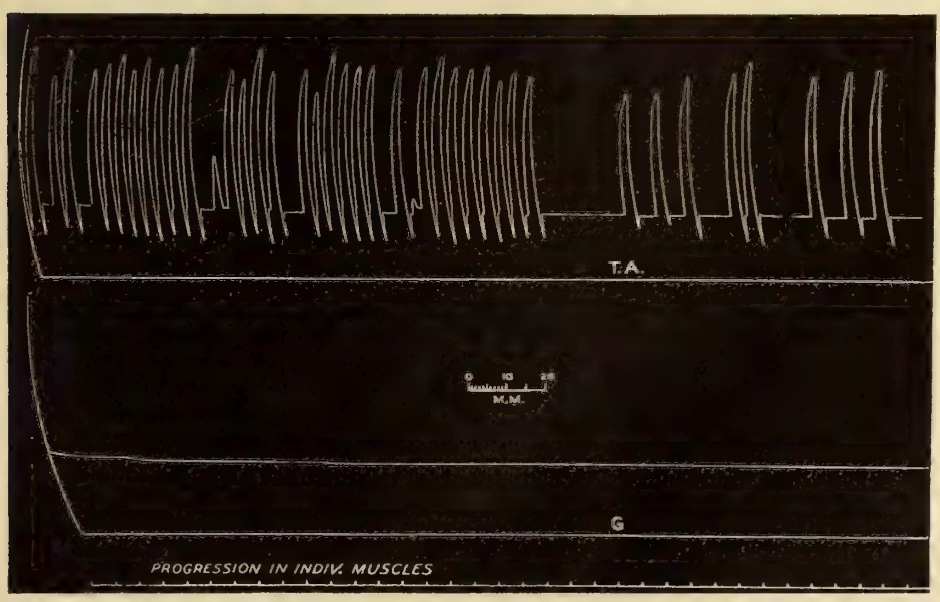

FIG. 7.—Experiment C, LX, record 107; 14.7.11.—Normal cat, narcosis progression registered in the isolated *tibialis anticus* and *gastrocnemius* muscles after motor paralysis of all the other muscles of both hind limbs. The upper tracing is that of the flexor (tibialis anticus), while the lower is that of the extensor (gastrocnemius). Rise of the curve denotes contraction and fall denotes relaxation of a muscle.

It will be observed that here the flexor beats are very well marked, but at this period of the experiment are somewhat irregular in extent and in rate of rhythm. No movement of gastrocnemius is registered.

may, however, persist for long periods, as long as 20 seconds. The beats may then reappear and again become suppressed, again to appear and persist. The beats at first may be more rapid than before the attainment of maintained flexion. At first they are synchronous in the two hind limbs, but as they slow in rate of rhythm they again become alternate.

In records obtained from the individual muscles at the ankle the effects of asphyxia have also been studied (fig. 8). Here the synchronism between the two hind limbs was not investigated, but with this exception the flexor showed the phenomena of asphyxia seen in the case of the intact hind limb. After the commencement of asphyxia there was little or no slowing of the rate of rhythm. The beats at once commenced to increase in height. The maximum was attained just before they began to increase in rate of rhythm. Thereafter the curve of minimum contraction of the flexor muscle began to rise (that is, the point of minimum contraction occurred at an ever greater level of maintained contraction). At the same time pauses disappeared from between the beats, their rate of rhythm progressively increased, and their extent progressively diminished. Their rate increased from about 1 cycle per second just before asphyxia, to about 3·4 cycles per second just before the complete effect appeared. The beats absolutely disappeared and left behind a state of maintained contraction of the flexor, which gradually increased.

Recovery from the effects of asphyxia has also been observed in the individual flexor muscle (fig. 8). The state of maintained contraction was then broken by groups of abortive beats which were of very small extent. Soon undoubted beats appeared. These were irregular, of slower rate than before, and of smaller extent.

The extensor played no part in the phenomena of asphyxia, or of recovery therefrom. Throughout it remained inactive.

IX. *Conclusions.*

That these movements of narcosis progression are strictly equivalent to the normal act of progression there can be no doubt.

In the rabbit the progression is almost invariably that of hopping—where the two hind limbs move synchronously in the same directions. In the phenomenon of narcosis progression of the rabbit the movements of the two hind limbs are also synchronous.

In the guinea-pig the progression is almost invariably that of bilateral alternation of movement in the two hind limbs. In the narcosis progression of that animal the movements of the two hind limbs have always been alternate.

In the cat the movement may either be that of bilateral alternation—as

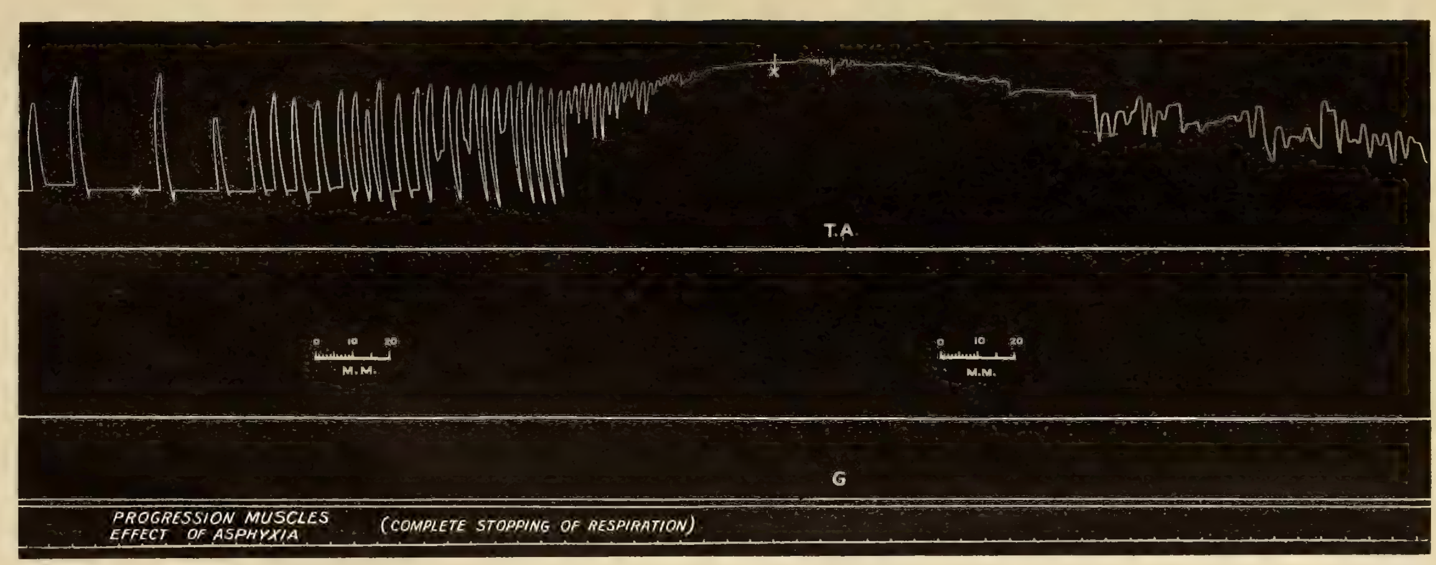

FIG. 8.—Experiment C, LX, record 107; 14.7.11.—Normal cat, from the same experiment as fig. 7—record of narcosis progression in the individual antagonists at the ankle. The effect of asphyxia ("X"–"X" approximate).

At the time of the commencement of asphyxia the flexor beats were very irregular. They then became more regular and very much more rapid. The pauses in flexor relaxation between the beats disappeared, the level of relaxation rose, and the complete effect was obtained when the beats disappeared and there ensued a state of maintained flexor contraction. This persisted and recovery gradually appeared. There were first some abortive beats, but when the beats really reappeared they were of extreme irregularity of extent.

in walking; or of very nearly perfect bilateral synchronism—as in the gallop. Its narcosis progression may take either form.

The great part played by the flexor muscle is a point of interest. It must be remembered that the movements of extension are much more affected by chemical narcosis than are those of flexion. It is perhaps because of this that when the individual muscles are examined the flexor alone appears to take part in the movements. It is probable that the extensor does take part in the better marked types of narcosis progression, although it is difficult to shew that it does. That muscle certainly takes part in the phenomena of progression which follow mechanical stimulation of the spinal cord and are extremely like the movements of narcosis progression. It also takes part in many instances of the rhythmic rebound phenomenon, which again is very similar to these two types of movement. But it is equally clear that the phenomenon of narcosis progression may appear when there is no evident contraction of the extensor at the ankle.

Again, the independence of the flexor centre is a point of great interest. The movements of narcosis progression may be present after decerebration. They may persist even after the isolation of the aboral part of the spinal cord by division in the lower thoracic region. The phenomenon of narcosis progression may therefore be conditioned by the lumbar centres alone, although it is probable that the higher centres in the cord and upper parts of the cerebro-spinal axis play an important secondary part in its conditioning. The movements may also occur after the destruction of the lower part of the spinal cord which contains the centres for the extensors of the ankle. But this does not necessarily mean that they would continue if all the extensor centres could be removed from the lumbar spinal cord. In this experiment there yet remained the extensor centres for the thigh. The movements may finally occur after the removal of a great part of one lateral half of the lumbar spinal cord. This all speaks for the independence of the lumbar centres.

It is true that in these experiments the movements were not observed after the de-afferentation of a hind limb in that limb. It must, however, be remembered that the movements of progression after division of the spinal cord have appeared in these circumstances, and so have the similar movements of rhythmic rebound. Perhaps the afferent proprioceptive impulses, which are almost certainly reïnforcing impulses and not essential to the act in the phenomenon of narcosis progression, play a greater part in the depressed state of the lumbar centres which is conditioned by the chemical narcotic. If so, it is of interest that the motor paralysis of a limb (accompanied by its de-afferentation, either actual or virtual) has little effect upon the movements of progression narcosis which occur in the other.

There is no space here to discuss all the points of interest raised by these phenomena, but I should like to mention one. The rhythmic act of progression resembles that of respiration in so complete a degree that it is difficult, if not impossible, to resist the idea that in all essentials they are the same, and conditioned in similar manners by similar mechanisms and by stimuli of similar sources.

There is a phenomenon of "voluntary breathing" just as there is one of "voluntary progression." But the former act tends soon to become involuntary; and although progression seems to be more under the influence of the higher centres, yet it, too, tends to become an involuntary act once started. The rhythmic movements of respiration seem essentially to be central; they appear to continue after the abolition of self-engendered impulses. In a similar manner it appears that the movements of progression in the lumbar centres may appear when self-generated proprioceptive impulses are excluded. Yet both respiration and progression are reïnforced by a peripheral self-regulative mechanism. The effects of asphyxia upon the two centres are again very similar; for in its effects upon the respiratory centre it first produces an increase in the amplitude of the respiratory movements. A state of maintained inspiration underlies this, the diaphragm may continue contracted to a certain extent even at the end of expiration. With an increase of the expiratory movements the inspiratory movements become small.

It looks as if there were here, and up to this stage in the asphyxia phenomena of respiration, a resemblance between the behaviour of the inspiratory centre and the flexor centre in progression. Is it possible that the flexor centre is strictly comparable to the inspiratory centre? That flexion = inspiration, and extension = expiration?

In the case of progression it is again of interest that asphyxia should produce an increase in extent and rate of rhythm of the movements. The act of progression if of sufficient speed itself may cause a certain degree of asphyxia. If this be not too great it will assist rapid movement through the environment. When carried to too great an extent the movements will be retarded, and the state of asphyxia will therefore be lessened. There is thus here possible a nice internal regulation of the speed of progression, and an optimum speed may be set for each resultant of the balance between the local peripheral and central factors, and the higher central and peripheral factors, which all influence the final centres which condition the act.

X. *Summary.*

1. Movements which seem exactly to resemble those of progression occur in some animals when subjected to general chemical narcosis.

2. In the rabbit these movements are synchronous in sense of direction in the two hind limbs. This corresponds with the normal hopping type of progression in these animals.

3. In the guinea-pig the movements which normally occur in narcosis are those of the scratch. If, however, the chemical narcosis be combined with novocain the resultant movements are those of progression. They are alternate in the two hind limbs, and thus resemble the movements of ordinary progression in the guinea-pig.

4. In the cat the movements which occur in narcosis are those of progression.

5. If the movements be examined in the two hind limbs they are found usually to be alternate. Rarely they are synchronous under normal circumstances—narcosis gallop.

6. If the depth of narcosis be gradually increased the movements of narcosis progression gradually fade out. If the depth of narcosis be decreased the movements progressively increase and then suddenly cease.

7. In either hind limb the movements—as examined at the ankle joint—consist of flexion succeeded by relaxation of flexion (extension), and with a pause in the posture of minimum flexion. Palpation of the tendons at the ankle shows that the flexors are active in this movement. It fails to demonstrate any extensor movement in the intervals of flexor contraction. This may, however, perhaps be present in the phenomenon.

8. The pauses may be long—of greater duration than the flexor beats—or they may be absent. In the latter case beat succeeds beat without intermission.

9. Occasionally in a record the movements may fail in one hind limb. It is then found that there is usually an exaggeration of the movements in the other. The beats become of greater extent and quicker than before. This augmentation gradually disappears if the beats in the other hind limb reappear. These, then, are at first smaller than usual, but soon attain their normal extent.

10. The phenomenon when present has been observed to continue after decerebration. In the lumbar centres it may also outlast rapid division of the spinal cord in the lower thoracic region. In the lumbar flexor centres it may also outlast a removal of the spinal cord aboral from them—in which the ankle extensor centres are cut off. The progression at one ankle may

also outlast removal of the lumbar cord of the opposite side of the body. The movements of narcosis progression have also been observed in a pair of antagonistic muscles at the ankle-joint after the motor paralysis of all the other muscles of both hind limbs. Immediately after the de-afferentation of one hind limb (by rapid division of the posterior spinal roots) the movements of narcosis progression have been found to be present in the normal hind limb, but have not then been present in the de-afferented limb.

11. When examined in a pair of individual antagonists at the ankle joint the movements of narcosis progression are found to be confined to the flexor, and then exactly to resemble the movements at the ankle examined in the intact limb.

12. Asphyxia produced by the complete closure of the trachea for a short period of time (15–40 seconds) produces a change in the movements. This is the same when examined either in the intact hind limb or in the individual flexor at the ankle. The flexor beats at first increase in extent and at the same time slow in rate of rhythm. They may sometimes be decreased in extent for a short time, and may even completely disappear. This phenomenon forms the first phase. When the beats have attained a maximum (after reappearance if they have previously disappeared) they commence to become quicker, and although they still continue to increase in height their relaxation is less complete than before, and their extent decreases. In this second phase of the asphyxia phenomenon there appears to be an increasing factor of maintained flexion. The beats become still more rapid and still smaller until they finally disappear. There is then left a state of marked maintained flexion (flexor contraction as seen in the flexor muscle).

13. If the movement in the two hind limbs is alternate at the point of commencement of asphyxia this alternation may change to synchronism as the beats become more rapid. This change is a gradual one. The apices of the beats in one hind limb gradually advance in temporal relationship to the apices of the beats in the other from the mid-point between these apices. This advance proceeds gradually until the apex of a beat in one limb synchronises with that of a beat in the other.

14. If the asphyxia be terminated at the point at which the beats disappear and a state of maintained flexion is left that state of maintained flexion may persist for a few or for many seconds, and may then be broken by the reappearance of beats. These then are fast and small, but become slower and larger in the reverse order to that which obtained during the establishment of the full asphyxia effect.

15. In narcosis progression the rate of rhythm is usually one of between

1 and 2 cycles per second. It may be as slow as 0·6 cycle per second; or as fast as 2·5. In asphyxia the rate which obtained at the point of commencement may be triplicated before the attainment of the complete effect. Thus a rate of 1 cycle per second may become one of 3·4. In normal narcosis progression the rate of rhythm may vary considerably in the same individual on different occasions.

Trichromic Vision and Anomalous Trichromatism.

By F. W. Edridge-Green, M.D., F.R.C.S., Beit Memorial Research Fellow.

(Communicated by Prof. E. H. Starling, F.R.S. Received November 15, 1912,—Read January 23, 1913.)

(From the Institute of Physiology, University College, London.)

Definitions.

A. *Trichromic Vision.*

The trichromic in my classification of degrees of colour-perception are those who have only three colour sensations—red, green, and violet. They see only three colours in the bright spectrum and describe it as consisting of red, red-green, green, green-violet and violet. They apply the designation red-green to the orange and yellow regions of the spectrum and green-violet to the blue region.

There are many degrees and varieties of trichromic vision (1, 2, 3, 4, 5). I have classified the colour-perception of individuals as dichromic, trichromic, tetrachromic, pentachromic, hexachromic, and heptachromic. This classification is made by estimating the number of definite colours seen in a bright spectrum, and the persons belonging to each class behave in every way as if they possessed the number of colour sensations indicated. On my theory of colour-vision each colour sensation is separate and distinct and not compounded of two or more fundamental colour sensations. For instance, there is the strongest evidence that yellow is a simple sensation (18, 24, 25, 26) and that spectral yellow light does not excite the red and green sensations.

B. *Anomalous Trichromatism.*

The term anomalous trichromatism is used in the sense of the Young-Helmholtz theory in which all colour sensations are supposed to be made up of different proportions of three fundamental sensations. A trichromat on this theory is therefore a person with normal colour-perception. An anomalous trichromat is a person who is supposed to have three fundamental sensations but they have not the same proportions as in the normal-sighted. Those are designated anomalous trichromats who, when making the equation $\lambda\,670 + \lambda\,535 = \lambda\,589$, use proportions of red and green different from the normal. At the same time the subjects of this abnormality object to the normal equation. Those who put too much red in the mixed colour are called red-anomalies and those who put too much green in the mixed colour green-anomalies.

Anomalous trichromatism was discovered by Lord Rayleigh (6), who stated that the colour vision is defective only in the sense that it differs from that of the majority. In 1904 Guttmann (8) stated that the anomalous trichromats were colour weak and described a number of symptoms similar to those given by me as associated with trichromic vision.

I then examined a number of persons with Rayleigh's apparatus (14) and found that many colour-blind persons, both dichromic and trichromic, can make a match which agrees in every particular with that of a normal sighted person.

I could find no evidence that colour weakness was necessarily associated with anomalous trichromatism. I examined 15 students from Newnham College on the same afternoon, the conditions being precisely the same for each. There was considerable variation in the observations and those who made an anomalous equation in every case strongly objected to the normal match. I could find no evidence of colour-blindness in any of those examined. All saw yellow in the spectrum. Of the 15 examined, five made the normal match exactly and one required slightly more green, the others more red in proportions varying in different cases; there was considerable difference between the two extremes, one requiring nearly twice as much red as the other in the mixed colour.

This year Lord Rayleigh kindly lent me his colour-mixing apparatus and I examined 100 women students, 25 belonging to the London County Council training college and 75 to University College. The last 75 were examined in precisely similar conditions. The illumination was incandescent electric light and the equation did not vary from day to day. All were examined with Lord Rayleigh's colour-mixing apparatus; 51 were examined by some kind of test for colour-blindness, and 36 of these were examined by my lantern. I have designated as "anomalies" those who, on an average of a number of observations, had a deviation of more than one whole division from the normal and did not agree with the normal equation. The colour-mixing instrument of Rayleigh was arranged so that 0 corresponded to full red and 25 to full green. Then by the laws of double refraction the exact proportions of red and green in any mixture can be ascertained. For instance, 12·73 corresponds to a ratio of intensity 1·061 green/red, and 10·371 to 0·5829 green/red. The other figures can be easily understood by remembering that a difference of one-tenth of a division corresponds to a difference of about 2½ per cent. in the ratio of intensities of red to green when the figures are in the neighbourhood of normal vision.

Out of the hundred examined, 86 made the normal equation or within one division on either side of it, 12 were anomalous trichromats, 10 being red-anomalies and 2 being green-anomalies.

Red-anomalies.				Green-anomalies.	
1.	1·5	6.	1·2	1.	3·1
2.	1·4	7.	1·8	2.	1·3
3.	1·2	8.	2·5		
4.	1·5	9.	1·3		
5.	1·3	10.	1·3		

Two others on an average of five observations appeared as anomalies (one 1·3 red, the other 2·0 green), but, as they both agreed with the normal equation, they do not come under the definition.

Excluding the last mentioned, who were to a certain extent colour blind, none of the anomalies were found to be colour defective. Of those who made the normal match 9 were found to be colour defective.

No. 1 of the green-anomalies was examined very carefully on three occasions; there was no evidence of colour-blindness; she passed my ordinary lantern test and also my triple lantern with ease and accuracy, and saw red and green through small apertures as far as I did. She also passed my bead test.

Examination with Spectrometer.—Pure yellow was isolated at λ 5770 to λ 5882. This is quite normal. The area of greatest luminosity was λ 5697 to λ 5795; this is considerably to the green side of the maximum of the normal luminosity curve. She marked out 18 monochromatic divisions in the spectrum. This is the normal number; she also named all the colours red, orange, yellow, green, blue, and violet correctly.

I have also examined a large number of men and find that when there is a large mean deviation there is colour weakness. The following case is instructive as an example of a high grade green-anomaly without any trace of colour weakness.

The observer was an assistant in the Chemical Laboratory of the Physiological Institute, University College.

Rayleigh Apparatus.—Shown red and yellow, named them correctly as red and yellow. The mean of seven equations was 17·3, the mean deviation 0·1.

The normal equation was 14·5. The mean deviation is very small.

Strongly objected to the normal equation ; said that the mixed colour was orange, and the simple, yellow.

Nagel's Test and Stilling's Test.—Passed both these tests with much greater ease and more rapidly than most normal-sighted persons.

My Lantern Test.—Passed easily.

The above tests were made in the presence of Prof. Starling and Dr. Homans.

Spectrometer.—

Region of greatest luminosity.........	λ 589–λ 605
„ pure yellow	λ 591–λ 596·5

My yellow region λ 583–λ 590 appeared greenish-yellow to him. This region inclines to orange-yellow to me.

Pure blue was λ 472–λ 476. Pure green, λ 510–λ 514.

Simultaneous contrast was not more marked than normal. Saw red below λ 780.

The following are the monochromatic regions marked out by him:—

	μμ.				μμ.		
1.	λ 780 1	Called by him	Red.	14.	λ 503 1	Called by him	Green-blue.
2.	λ 626 1	„	Orange.	15.	λ 497 1	„	„
3.	λ 613·5 1	„	Orange-yellow.	16.	λ 491 1	„	Blue.
4.	λ 605 1	„	Yellow.	17.	λ 483·5 1	„	Deep blue.
5.	λ 597 1	„	Greenish yellow.	18.	λ 475 1	„	Violet-blue.
6.	λ 590 1	„	„ „	19.	λ 466·5 1	„	Blue-violet.
7.	λ 579 1	„	Yellow-green.	20.	λ 457 1	„	„
8.	λ 567 1	„	„	21.	λ 447 1	„	Violet.
9.	λ 558 1	„	„	22.	λ 435 1	„	„
10.	λ 541 1	„	Green.	23.	λ 426 1	„	„
11.	λ 523 1	„	Blue-green.	24.	λ 417 1	„	„
12.	λ 516 1	„	„	25.	λ 411 1	„	„
13.	λ 509 1	„	Green-blue.		λ 407		

It will be noticed that the region regarded by the normal-sighted as orange-yellow is named and seen by him as greenish yellow. This gives an explanation of the anomalous trichromatism. If the region to be matched appears greener than usual, it will obviously require more green and less red in the mixed colour.

These were the results of single observations ; the available time would not admit of more and they clearly confirm the other tests.

THE RELATION BETWEEN TRICHROMIC VISION AND ANOMALOUS TRICHROMATISM.

Anomalous trichromatism should be clearly defined as the condition in which anomalous matches are made by a person who refuses to accept the normal match. Much confusion exists on this point; a person who agrees with the normal equation cannot be regarded as an anomalous trichromat even though he agrees at the same time with the anomalous matches. This is only evidence of colour weakness, inasmuch as both equations are regarded as satisfactory. There are many anomalous trichomats who are not colour weak and there are many trichromics who make absolutely normal equations. Trichromic vision in my classification is therefore not synonymous with anomalous trichromatism. There are also persons who will make the normal equation in one set of circumstances and anomalous equations in another (14). There are also those who will make normal equations when the red employed is λ 670 but will make an anomalous equation with a red of larger wave-length, as for instance λ 690, putting twice as much red in the mixture compared with the normal equation in similar circumstances (16). Anomalous trichromatism when too much red is put in the mixed colour may correspond to defect in the perception of certain red rays, namely those employed in the mixed colour. I have shown (5) that when there is shortening or much defect in the perception of red the junctions of the other colours are shifted towards the violet end of the spectrum. The yellow, therefore, corresponding to the D line, is seen as a much redder colour than the normal, and if we consider that the green is similar to the normal it is obvious that more red will be put in the mixture than by the normal-sighted. This shortening of the spectrum may be associated with normal vision in other respects or with any degree of defective colour differentiation, that is to say, it may be associated with dichromic, trichromic, tetrachromic, pentachromic, hexachromic or heptachromic vision. A similar condition is also found for the violet end of the spectrum. It is obvious that a man, who has shortening of the red end of the spectrum or defect in the perception of red, is colour weak as far as red is concerned. Unless, however, he has defective hue perception he may make no other error than that directly connected with the defective perception of certain red rays. It is different with those who make an anomalous match in which too much green is put in the mixed colour. As found by Rayleigh (6), Köllner (20), v. Kries (10), Nagel (13), and myself (14), a man may make an anomalous match without presenting any other colour defect. I have found 25 per cent. of men to be more or less colour weak, and it is, therefore not surprising that anomalous trichromatism is frequently associated with colour weakness. The colour weak are also particularly liable to fail in making an equation, but in addition to making the anomalous equation they are in most cases satisfied with that of the normal. Anomalous trichromatism cannot be due to the diminution of a green sensation in the sense of the Young-Helmholtz theory. Apart from the fact that I have shown that yellow is a simple and not a compound sensation (18, 24, 25, 26), there would be no reason why more green should be required in making the compound yellow, since the simple yellow would also contain less of the hypothetical green sensation. If whilst the yellow remains as in the normal the sensitiveness to green light were diminished or to red increased we should have an explanation of the facts. Schuster (19) found that the position selected as pure yellow was the same with the green-anomaly as with the normal-sighted. Whilst there are red-anomalies who show weakness for red, there are others who do not, and this may be explained by an increased sensitiveness to green whilst the red and yellow remain as in the normal.

SUMMARY.

1. Trichromic vision is not synonymous with anomalous trichromatism.

2. Many persons with otherwise normal colour-perception make an anomalous equation.

3. Many colour-blind persons (dichromics and trichromics) make an absolutely normal match with no greater mean deviation than the normal.

4. Colour weakness is not characteristic of anomalous trichromatism but of trichromic vision.

5. Anomalous trichromatism and colour weakness are not synonymous.

6. A large mean deviation indicates colour weakness.

7. Anomalous trichromatism appears to be due to an alteration in the normal relations of the response to the three colours (lights) used in the equation. If the eye be more or less sensitive to one of the components of the mixed colour whilst the other has its normal effect, an anomalous equation will result. An anomalous equation will also result when the yellow is more allied to green or red than is normal.

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A Preliminary Note on a New Bacterial Disease of Pisum sativum.

By Dorothy M. Cayley, Research Student, John Innes Horticultural Institution, Merton, Surrey.

(Communicated by W. Bateson, F.R.S. Received November 19, 1912,—Read January 23, 1913.)

Investigations have been carried out this year at the John Innes Horticultural Institution to elucidate the nature of a disease which affects culinary peas (*Pisum sativum*).

The disease, in this district at all events, is a serious one, killing a large proportion of the crop, but I have no information as to its prevalence in other parts of the country. I have succeeded in proving that the disease in culinary peas is caused by a large bacillus which exhibits a peculiar feature, inasmuch as it is transmitted in the interior of the seeds of the plant. As far as I am aware no analogous instances are known. Owing to the work of Chamberland (1879), Lehmann (1889), Laurent (1891), and others, it has been definitely proved that not only is the interior of a normal seed sterile, but also beans and peas taken under sterile conditions from healthy pods are free from bacteria.

A very large rod-shaped bacillus has been isolated from the stem of the living pea plant and from the centre of the cotyledons of the pea. The life history of the organism is complicated by involution-forms and a zoogl&oelig;al stage. In the rod stage the bacillus is Gram-positive, non-acid-fast, very motile when young but enveloped in a capsule when at rest. It varies considerably in size according to the amount of water, food material, and other conditions. It grows well on acid (1 per cent. normal HCl), alkaline (1 per cent. normal NaOH), and neutral pea agar agar, forming small circular, pale buff, translucent, watery colonies on the surface of the medium, and when submerged the colonies are deeper in colour, opaque and lens-shaped. The colour of the colonies varies according to the medium used. Under certain conditions the colonies may have a decided orange tint. This was especially noticeable in an impure culture into which a spore of Penicillium had been introduced. The bacterial colonies immediately round the Penicillium were both larger and of a deeper tint than in other parts of the Petri dish where no fungoid growth occurred.

This orange tint has also been observed in badly diseased cotyledons after germination, but does not necessarily occur in all cases. Further elucidation of this point is necessary.

No growth has so far been observed on lactose pea agar agar.

In liquid peptone beef broth the rods grow to a great length and are strung together in chains.

The organism occurs in the phloem, cambium, medullary rays, and occasionally in the pith of the stem, also in the parenchyma of the vascular bundles which run along the mid-rib of the pod, in the tissue of the funicle and cotyledons.

In the very young plant grown in sterile sand the bacillus has been found in the primary ground-tissue of the radicle inside the pericycle, and in the young phloem and cortical tissues of the shoot.

The general symptoms are as follows:—In mild cases after germination the shoot can develop normally, but in bad cases it is frequently abortive, brown and dead at the tip, and laterals grow out prematurely to take the place of the main shoot. Quite early in the development of the plant, when the plumule is from half an inch and upwards in length, light brown longitudinal streaks can be seen on the stem and root, and the first leaves are often brown at the tip. These streaks develop later into slits. In very bad cases little or no germination takes place. After this stage no further definite signs are noticeable till about the flowering period. Then the development of the disease depends a good deal on external conditions. If the weather is warm and dry, and the plants are growing vigorously, the disease develops rapidly, and in a few days the plants become unhealthy and change colour. The stem turns slightly brown, and looks somewhat water-soaked. Brown longitudinal streaks appear at the base of the petioles on either side of the rib of the stem, which is continuous with the mid-rib of the leaf. The streaks split open and dry out. The collar may be badly disorganised. The leaves become spotted, streaked and yellowish in colour, and if the disease is progressing rapidly the younger portions of the plant show discoloration, and fail to develop properly.

Except in bad cases the plants grow to full height, and can flower and set a certain amount of seed, but on examination the cotyledons of the seeds of a diseased plant show brown discoloration, which may be limited to a mere spot in the centre of each cotyledon, or, on the other hand, nearly the whole of the cotyledon may be involved. In the latter case there is often a cavity in the centre of the cotyledon.

Sections of the diseased cotyledon show large numbers of bacilli in various stages of development in the cells and intercellular spaces.

The bacillus works its way into the intercellular spaces and then breaks into the cells. There the nucleus is often attacked, the cytoplasm destroyed, and the cells collapse, thus forming rents in the tissues.

There is considerable evidence to show that the bacillus passes up the plant through the tissues above mentioned, through the funicle, and probably the micropyle into the young developing seed. If one pea is diseased all the other peas in the same pod are diseased to an equal extent. The disease is chiefly spread by the seed, but fresh infection may take place through the soil.

Inoculation experiments were carried out in the open, but little stress can be laid on the results, as the disease was so prevalent throughout the experimental plot. Pea plants grown in heated soil in boxes, and inoculated just above the ground, when the plants were about 1 foot in height, showed no disease, whereas, in the open, seven out of ten inoculations on the stem just below the youngest unfolding leaf were successful.

Further inoculation experiments are necessary, but the above results tend to show that the bacillus can only penetrate very young tissue. This is supported by the fact that large numbers of the bacilli have been found in the inner tissues of the radicle when only about half an inch long.

Further investigations are in progress.

In many respects the symptoms resemble those of the formidable disease of sweet pea (*Lathyrus odoratus*) known as "streak." This disease has been held to be due to *Thielavia basicola*, but, in view of these observations, that conclusion seems very doubtful, and I may add that, in the stem of diseased sweet peas, I have already found bacteria like those here described.

On the Manganese Content of Transplanted Tumours.

By F. Medigreceanu, M.D.

(Communicated by Sir J. R. Bradford, K.C.M.G., Sec. R.S. Received November 21, 1912,—Read January 23, 1913.)

(From the Laboratory of the Imperial Cancer Research Fund.)

The occurrence of manganese in plants is well known. Its quantitative distribution and biological significance have been carefully studied from many points of view. A few examples of the more recent, especially experimental work, illustrating some of the biological properties of this metal, may be mentioned. Bertrand* showed that there exists a close relationship between the activity of vegetable oxydases and the amount of manganese present. In a series of very exact experiments with *Aspergillus niger*, the same author demonstrated that the presence of manganese is necessary to the formation of conidia of this mould,† and also that the rapidity of its growth may be largely influenced by the quantity of manganese added to the culture medium.‡

The study of manganese in animals is far less advanced than in plants. Since the food-stuffs contain manganese, it is obvious that this element is continuously introduced into the animal body. The detection of manganese in animal tissues has been the subject of repeated investigation during the last 70 years. The conclusions, however, which the earlier authors have drawn are very contradictory, undoubtedly attributable mainly to the insufficiency and the defects of the methods and the technic used for the detection and estimation of this element.§

Recently Bertrand and Medigreceanu applied Bertrand's colorimetric method for estimating the manganese in organic substances‖ to an extensive analytical study of this metal in normal animals. By means of this method manganese can be estimated even when present in very small quantities, 2/1000 mgrm., and with an error not exceeding 10 per cent.

Manganese was thus found to be a normal constituent of the organism throughout the animal kingdom.¶ The invertebrates usually show relatively much larger quantities of manganese than the vertebrates, and of the vertebrates the mammals contain the smallest amounts—a few hundredths of a milligramme per 100 grm. of the total weight of the organism—while the birds, reptiles, batrachians, and fishes show 5–10 times as much.

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* G. Bertrand, 'Comptes Rendus,' 1897, vol. 124, p. 1032.

† 'Bull. Soc. Chim. France,' 1912, Ser. 4, vol. 11-12, p. 494.

‡ *Ibid.*, p. 400.

§ See Bertrand and Medigreceanu's article, 'Bull. Soc. Chim. France,' 1912, Ser. 4, vol. 11-12, p. 656.

‖ 'Bull. Soc. Chim. France,' 1911, Ser. 4, vol. 9, p. 361.

¶ See Bertrand and Medigreceanu, 'Comptes Rendus,' 1912, vol. 154, pp. 941, 1450 ; 1912, vol. 155, p. 82.

The quantitative distribution in the different organs, tissues, and animal products, especially of the higher classes that have been studied, is very interesting. The blood, for example, contrary to the claims of most previous investigators, has been found to contain much smaller amounts of manganese than sometimes admitted, usually only a few hundredths of a milligramme per litre. The hæmoglobin of horse blood contains no manganese. Of the organs and tissues of principal functional importance higher manganese values have been met with in the liver (0·265–0·416 mgrm. per 100 grm.) and in the kidneys (0·063–0·238). Lower values are found in the muscular tissue ($<$ 0·005–0·018), the nervous tissue ($<$ 0·005–0·036), and the lungs (0·006–0·023). The organs of the birds are generally richer in manganese than those of mammals, and the highest value obtained has been for the oviduct of birds (0·786–2·201).

It may be mentioned that the grey matter of the ox brain is much richer (0·022) in manganese than the white ($<$ 0·005), and also that, in general, the heart and muscles of the tongue contain larger amounts of this metal than the trunk muscles and the muscles of the extremities.

Among the organs or tissues of minor functional importance, the hair, plumage, and nails contain relatively large amounts of manganese (0·111–3·214).

The milk is very poor in manganese, although apparently richer than the blood. In the egg-white (fowl and duck) they were unable to detect this metal, even when analysing 100 grm. of the fresh substance. The yolk seems to contain all the manganese present in the egg.

Considering the ubiquity of manganese throughout the animal kingdom, and its remarkable distribution in the various tissues, these authors emphasise the importance that it probably has as a catalytic agent of living matter. Again, the wide differences shown to obtain between the amount of manganese found in plants and in animals is an observation which may have considerable importance. The quantities of manganese present in the various organisms and tissues may very well be taken into consideration in studying the problems of the origin of species, as well as those of biochemical adaptation to the medium, in interpreting the influence of vegetarian and flesh diets, and finally in drawing deductions as to the nature of the physiological soil.

In connection with Bertrand and Medigreceanu's work on manganese, it seemed of interest to study its occurrence and quantitative distribution in tumours. The transplantable tumours (mouse, rat, dog) were chosen for the purpose. These kinds of tumours are at present the best known as regards their biological and morphological properties, and the most suitable for an exact and rapid orientation.

The tumour strains analysed belong to both the principal morphological groups—carcinoma and sarcoma. Each strain shows different morphological and biological properties.*

Technic.

As already mentioned in the introductory part, the estimation of manganese was made by Bertrand's colorimetric method. It consists essentially in converting the manganese present in the sulphate ash of the organic substance into permanganic acid, in oxidising the ash dissolved in concentrated nitric acid with potassium persulphate in the presence of silver nitrate, and in comparing the intensity of its rose-pink to violet colour with the colour of standard solutions of the same acid prepared in a similar way. The details of the method were followed exactly as given by Bertrand and Medigreceanu.†

Of the tumour tissue to be analysed quantities not exceeding 100 grm. were first dried at 100° C. and then incinerated at the lowest possible temperature, using sulphuric acid for the destruction of the final traces of carbon. The sulphate ash was then dissolved in concentrated hydrochloric acid, again treated with a little sulphuric acid, and finally heated until the appearance of white fumes of sulphuric acid denoted the absence of hydrochloric acid. The residue was then dissolved in 10 c.c. of 25-per-cent. nitric acid, and if necessary the undissolved part of the ash allowed to precipitate. A few drops of 10-per-cent. silver nitrate was then added to the clear solution, the tube warmed, and its contents oxidised with a few decigrams of potassium persulphate.

The greatest care was always taken to avoid introducing impurities containing manganese into the samples for analysis, and pure reagents were used throughout.

It may also be mentioned that the small quantities of blood contained in the tumours do not influence the analytical results, for it was found that 25 grm. of mouse blood treated in the same way did not show any visible trace of manganese. This fact fully agrees with the previous findings of Bertrand and Medigreceanu, who observed only traces of manganese in larger

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* Full details on the tumours analysed may be found in the 'Fourth Scientific Report on the Investigations of the Imperial Cancer Research Fund,' London, 1911, Taylor and Francis.

† 'Bull. Soc. Chim. France,' 1912, Ser. 4, vol. 11–12, p. 656.

Mouse Tumours.

Tumour strain.	Age of tumours, in days.	Weight of the fresh material examined, in grammes.	Milligrammes of manganese.		Remarks.
			Found in the sample.	Per 100 grm. of the fresh substance.	
Adeno-carcinomata and Carcinomata.					
63 (1) 65 D	28	60	0·006	0·01	39 mice. Average weight: mice 17 grm., tumours 1·6 grm. Tumours generally cystic.
(2) 65 D 66 D 73 A	18–50	21	0·002	0·009	10 mice. Average weight: mice 15·4 grm., tumours 2·1 grm.
91 (1) 30 B 43 A	28–82	28	0·003	0·01	10 mice. Average weight: mice 18 grm, tumours 2·8 grm. Necrotic and cystic.
(2) 44 A	19	47	0·004	0·008	23 mice. Average weight: mice 16 grm., tumours 2 grm.
199 42 A 43 A 44 A	29–69	22	0·003	0·013	11 mice. Average weight: mice 15·8 grm., tumours 2 grm.
37 54 H 50 M 61 F 65 D 69 D	48–130	46	0·006	0·012	13 mice. Average weight: mice 17 grm., tumours 3·5 grm.
100 50 E 51 E 51 F	44–68	20	<0·002	<0·01	13 mice. Average weight: mice 16 grm, tumours 1·5 grm. Large cysts, containing opalescent liquid.
286 32 B 33 A 34 A	22–69	14	<0·002	<0·014	12 mice. Average weight: mice 17 grm., tumours 1·1 grm.
Sarcomata.					
100 75 B	13	66	0·003	0·004	24 mice. Average weight: mice 15 grm., tumours 2·7 grm.
92 53 A 54 A 58 A 60 A	23–180	26	<0·002	<0·007	13 mice. Average weight: mice 22 grm., tumours 2 grm.

Rat Tumours.

Tumour strain.	Age of tumours, in days.	Weight of the fresh material examined, in grammes.	Milligrammes of manganese.		Remarks.
			Found in the sample.	Per 100 grm. of the fresh substance.	
			Carcinoma.		
F.R.C. 75 A 78 A	42–105	18	<0·002	<0·01	5 rats. Average weight: rats 64 grm., tumours 3·6 grm. Very necrotic.
			Sarcoma.		
J.R.S. (1) 77 A	73	50	0·003	0·006	1 rat. Weight: rat 107 grm., tumour 52 grm.
(2) 76 A 78 A	52–90	80	0·005	0·006	2 rats. Average weight: rats 83 grm., tumours 41 grm.
(3) 80 A	19	55	0·005	0·009	16 rats. Average weight: rats 74 grm., tumours 3·4 grm.
(4) 75 A 77 A 79 A	39–123	(*a*) 50 (*b*) 86	0·005 0·007	0·01 0·008	5 rats. Average weight: rats 98 grm., tumours 30 grm.
			Dog Tumour. Lympho-Sarcoma ?		
Generation 40 A	27	22	<0·002	<0·003	3 dogs. Average weight: tumours 7 grm.

quantities of the blood of several mammals and birds. It serves also as a control to the purity of the reagents used. The analytical results in the several tumour strains examined are shown in the adjoining table.

Summary and Conclusions.

As a general conclusion it may be stated that the quantities of manganese found in transplanted mouse and rat tumours, whether carcinomata or sarcomata, and also in the so-called lymphosarcoma of the dog, are very small —they vary between 0·004 and 0·012 mgrm. per 100 grm. of the fresh material.

In order to obtain an idea of the comparative amounts of manganese contained in the normal mammary gland and the epithelial tumours derived from it, two manganese estimations were made of normal mouse mamma. In the first, 15 grm. of lactating mamma were analysed. Only 0·004 mgrm. was found, *i.e.* 0·026 mgrm. manganese per 100 grm. For the second determination, 14 grm. of resting mamma were incinerated. The amount of manganese present was 0·002, *i.e.* 0·014 mgrm. per 100 grm. Though the comparison be not strictly exact, nevertheless the figures obtained allow this general conclusion to be drawn, that the epithelial transplantable mouse tumours developing in the mammary gland do not contain a larger amount of manganese than their normal mother tissue.

Furthermore, there are not very marked differences in the percentage distribution of manganese between carcinomata and sarcomata. In connection with this observation it may be also mentioned that the carcinoma and sarcoma strains of the mouse tumour "100" do not exhibit appreciable differences in their manganese content.

The Influence of the Resilience of the Arterial Wall on Blood-Pressure and on the Pulse Curve.

By S. Russell Wells and Leonard Hill, F.R.S.

(Received November 29, 1912,—Read February 6, 1913.)

This communication is the result of two independent but converging lines of research. It is well known that when a fluid is driven with a rhythmically varying pressure through a sufficient length of a distensile elastic tube, the pressure at the exit loses its rhythm and becomes constant and the flow continuous, whereas if the tube is rigid, the pressure at the outlet varies as that at the inlet (less the change due to friction) and the outflow is intermittent.

Since the arteries are distensile elastic tubes and the blood is rhythmically forced into them by the heart, it follows that the curve of blood-pressure must be altered to a greater or lesser degree by the distensibility and elasticity of the arterial wall.

We use the term resilience in this paper to express the ease with which an elastic tube distends with a rise and recoils with a fall in pressure of the contained fluid; thus, a rubber tube with a wall 0·2 mm. thick is more resilient than one with a wall 0·4 mm. thick, the thinner, more resilient tube yields with the rise and recoils with the fall of pressure more than the "harder," thicker walled, less resilient tube. A glass tube in this sense has no resilience, and the same may be said of rubber pressure tubing.

As the arterial wall contains muscle its resilience will be altered by a more or less contracted state; as the degree of contraction and resilience may vary locally it is to be expected that the curve of blood-pressure may also vary, *e.g.* in the brachial and femoral arteries. Further, as the peripheral resistance in any area may alter the tension of the arterial wall, its resilience may vary without any change in the muscular state of the arterial wall.

Observations made by one of us (L. H.) with W. Holtzman and Martin Flack, and later with R. A. Rowlands,* on cases of aortic regurgitation placed in the horizontal position, have shown that the systolic pressure is much higher in the leg than in the arm, *e.g.* 100–150 mm. of mercury higher, and so characteristic is this difference that it is a diagnostic sign of the condition. Thomas Lewis found that the same held good in the case of a dog in which he had experimentally rendered the aortic valves incompetent one month previous to taking the observations.† His measurements were recorded by means of cannulæ placed in the arteries and connected with Hürthle manometers. Hürthle* and others have recorded previously higher readings of pressure in the femoral than in the carotid artery of the dog. Tigerstedt ascribed these to reflection of the primary pulse wave without change of sign and addition of the reflected to the primary wave in the femoral artery.† The difference of the systolic pressures in the arm and leg in aortic cases was ascribed by L. H. and his co-workers to the better conduction of the systolic wave crest in the leg arteries, which were assumed to be in a more contracted and harder state.

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* 'Heart,' 1909, vol. 1, p. 73; and 1912, vol. 3, p. 219.

† 'Heart,' 1912, vol. 3, p. 222.

This view was confirmed by experiment, for it was found, on placing the legs and buttocks of the patients in a hot bath, the difference between the readings of arm and leg arteries was abolished, and this was ascribed to the expanding and softening of the contracted walls of the latter. Also, in the case of healthy young men placed in the horizontal posture, while it was found that the leg and arm readings of systolic pressure were normally the same, these were rendered temporarily unequal after the subjects had run twice up and down a long flight of stairs (particularly if the arm were placed in hot water beforehand); the heart was thereby made to beat forcibly, while the leg arteries became more contracted, so the crest of the wave was better conducted in them than in the arm arteries. By placing one arm in hot water, it was found possible to render the reading different in the two arms, even in the resting subject, much more so after a short period of violent exercise. If the wrist alone were placed in hot water, the radial gave a lower reading than the brachial, but if the elbow were placed in hot water, readings of brachial and radial were equal, both being lower than in the other and cooler arm; bandaging the hand tightly made no difference to the reading.

The conclusion arrived at was that the inequality was due to an altered condition of the arterial wall and not to diminished peripheral resistance, and these experiments led to the conception that the nature of the arterial wall affects the conduction of the systolic wave, and that the blood-pressure, as ordinarily measured by a sphygmometer, by the method of obliteration of the pulse, depends not only on the pressure wave produced by the heart, but also on the effect on this wave of the arterial wall, a new factor which has not hitherto been taken into account.

A difference of pressure between the arm and leg readings has been noted by several observers in cases where the arteries are thickened and hardened as in old people. This difference has been ascribed to an error in the

* 'Arch. f. d. ges. Physiol.,' vol. 47, p. 32.

† 'Lehrb. d. physiol. des Kreislaufes,' 1893, p. 352.

readings due to the thickened artery resisting compression, just as an empty rubber tube does.

One of us (L. H.) and Martin Flack have found that such differences of pressure are lessened by keeping on the pressure of the armlet, and lowering and raising it so as to take several readings of: (1) the reappearance; (2) the disappearance of the pulse. Our explanation is, that the artery cut off from the blood relaxes and softens, and therefore the crest of the systolic wave is diminished. It has been shown by Bayliss that compression of an artery is followed by vascular dilatation in the area cut off from the blood.

In many of these cases the force of the pulse is irregular; now and again an extra large systolic crest forces its way beneath the armlet, and such large waves are better conducted by the leg arteries, just as happens in the case of aortic regurgitation.

By means of a circulatory schema, in which two lengths of artery are inserted, one to be compressed, the other to be palpated (the latter gave the index, the disappearance of the pulse), it was easy to demonstrate that the systolic pressure is read more accurately when the palpated artery is made tense (produced in this schema by obstructing the outflow by means of a mercury valve) than when it is soft. In the first case the readings of systolic pressure taken in the pump and in the artery are the same, in the second case the reading taken in the artery is lower.

In the living animal with its vasomotor nerves, and pressure changes of rapid rate, and output of the heart varying from second to second, it is extremely difficult to study exactly the effect of the various factors on the character of the pulse curve, for one cannot vary at will one of these factors without affecting the others. From these considerations it appeared desirable to one of us (S. R. W.) to investigate the subject by means of non-living elastic tubes. Halls Dally and K. Eckenstein have assisted in this research, which will be published in full later.

After considerable experiment an apparatus was devised, by means of which fluid at a known rhythmically changing pressure could be passed (*a*) through elastic tubes of the same calibre, but with walls of various known thicknesses; (*b*) through various lengths of the same tube, and (*c*) keeping to the same tube, the absolute pressure could be varied, while maintaining the same difference between the systolic maximum and the diastolic minimum, or this difference could also be varied at will.

The tubes used in the experiments were various lengths of rubber tube all of the same internal calibre, but with walls of 0·8, 0·6, 0·4, and 0·2 mm. thickness. The pressure variations of the fluid before flowing through the resilient tube and at the end of it were recorded by Hürthle's manometer.

It was found when the same resilient tube was used, but the diastolic pressure of the entering fluid varied, keeping the interval between the systolic and diastolic pressures as far as possible the same, that the higher the pressure and consequently the more the resilience of the tube was brought into action by stretching, the nearer together were the diastolic and systolic pressures at the end of the resilient tube. In other words the smaller was the amplitude of the pressure waves, and the more closely did the pressure approach to a continuous one. As an instance, the following experimental results may be cited, working with 30 cm. of a rubber tube, the walls of which were 0·8 mm. thick and recording the pressures in millimetres of Hg.

Entering pressure.			Pressure at end of rubber tube.			Difference between initial and end pressure.	
Systolic.	Diastolic.	Difference.	Systolic.	Diastolic.	Difference.	Systolic.	Diastolic.
145	50	95	120	60	60	−25	+10
184	86	98	152	104	48	−32	+18
220	125	95	188	148	40	−32	+23

The same general results followed, no matter what the thickness of the wall of the tube experimented on might be, and no matter what its length, but the difference in the case of the thinner walled tubes was even more striking.

Working with a rubber tube 30 cm. long, and with walls 0·2 mm. thick, with an entering pressure of 78 mm. of Hg diastolic and a 148 mm. systolic, an almost continuous pressure of 104 mm. diastolic and 107 mm. systolic was obtained at the end of the resilient tube.

With raised pressure not only was the curve of less amplitude, but its form also was altered, the top becoming flattened and the dicrotic wave less marked, indeed it took on the characters which have frequently been described as occurring in the sphygmograms of cases of high blood pressure.

In order to test the correctness of the supposition that as the general level of pressure was raised the resilience of the wall was increasingly brought into play, a series of experiments was carried out, using the same initial pressures and the same thickness of tube wall, but varying lengths of tube. It was found that lengthening the tube had the same effect of approximating the systolic and the diastolic pressures and making the curve take on the characters of a "high pressure" sphygmogram. For instance, using a tube with walls 0·8 mm. thick, the following results were obtained:—

Length of tube in cm.	Entering pressure.			Pressure at end of rubber tube.			Difference between initial and end pressure.	
	Systolic.	Diastolic.	Difference.	Systolic.	Diastolic.	Difference.	Systolic.	Diastolic.
15	160	40	120	126	60	66	−34	+20
30	160	42	118	118	74	44	−42	+32
60	117	40	117	108	78	33	−49	+38

The same initial pressure differences were then tried on tubes of the same length and calibre, but with walls of different thicknesses, namely, 0·8, 0·6, 0·4, and 0·2 mm., when the same sort of results were obtained, viz., the thinner and consequently the more resilient the tube, the more was the systolic pressure lowered and the diastolic raised by passing through the tube, that is, the nearer the resultant curve approached a straight line.

It was quite remarkable to observe how with an entering pressure such as 160 mm. systolic and 40 mm. diastolic, a curve in the 0·8 mm. tube would have all the characters of a low pressure sphygmogram, great amplitude, sharp rise and fall and very well marked dicrotic wave, while with exactly the same entering pressure the curve in the 0·4 mm., and more so in the 0·2 mm., took on all the characters of a high pressure sphygmogram, slow rise, flat top, slow fall, and slightly marked dicrotism.

L. H. and Martin Flack have since found that the introduction of, say, 6 cm. of cat's carotid artery in place of an equal length of pressure tubing alters the characters of the pulse curve from a low to a high pressure curve. The experiments demonstrating this will be published in full later.

From these experiments conducted by S. R. W. and those of L. H. it seems legitimate to draw these conclusions: the form of curve obtained by a sphygmograph or other instrument recording the pulse is the resultant of two factors, the blood-pressure variations produced by the heart and the resilience of the arterial wall, using the term resilience in the sense defined above.

Much at times has been made of the supposed influence of reflected waves on the pulse curve. It is the resilience of the wall which we believe to be the important factor in modifying the curve, and not the reflection of waves from the periphery.

The blood-pressure measured in any artery by the sphygmometer is likewise the resultant of these two factors, and the measurement does not necessarily give us the full systolic pressure produced by the heart; much of the force is spent in dilating a soft distensile artery. Further, since the character of the flow in an artery largely depends on the resilience of its walls, it is obvious that, the more resilient or yielding are those supplying any part, the more closely will the blood stream at the threshold of the capillary area supplied approach a uniform pressure (roughly the mean between the systolic and diastolic pressures, less, of course, what has been lost by friction), while the harder or less resilient the arterial wall, the more closely will the variations approach those in the aorta.

Now all the arteries, and to a greater extent the arterioles, are contractile, and under the influence of the nervous system, and with an increased tone or contraction, they become not only narrower as to lumen, but also thicker as to wall, that is less resilient, so it may happen that the organism can with the same heart force vary the pressure at the threshold of a particular capillary area between an intermittent pressure with a high systolic beat and an almost continuous one, with a lower systolic pressure. In the one case there would be a hammer-like percussive wave beating open the capillaries, the blood would be hammered in ; in the other there would be a more continuous pressing in of the blood at a lower tension.

It may be that narrowing of lumen and lessened total flow goes with the more percussive wave due to hardening, but this does not necessarily follow, for it is possible that a tightening of the muscular coat, and a lessening of the resilience, may take place before actual narrowing occurs. It is further possible that the great arteries and the arterioles act differently, or independently in some cases.

We advance the view that the throbbing and capillary pulse observed in an acutely inflamed area is due to an increased tone of the arterial walls, a lessening of their resilience ; this throbbing is often relieved by hot fomentations which act by relaxing the contraction of the vessel walls. In cases of aortic regurgitation the hammer-like pulse propelled through the harder leg arteries secures to the legs an adequate supply of blood, compensating as it does for the diastolic fall due to the regurgitation ; there, again, hot water baths relax the arterial wall.

One of us, L. H., with Martin Flack,* has shown that in the case of the salivary gland each alveolus is surrounded by a tough membrana propria which resists expansion and allows the secreting cells to draw fluid from the capillaries and raise the secretory pressure to almost double the height of the arterial blood-pressure, without obliterating the surrounding capillaries or interrupting the venous outflow. Under such conditions the veins are narrowed, by the expansion of the alveoli up to their limiting membranes, and the blood vessels, arteries, capillaries, and veins form a system of rigid vessels with a rapid rate of flow, the pulse even coming through into the veins, the whole gland feeling tense to the touch. Increased hardness of the arteries, supplying such an active organ, permits the full force of the systolic wave to come into play, and insures a flow of blood in the face of the increased osmotic pressure and swelling of the tissues.

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* 'Roy. Soc. Proc.,' 1912, B, vol. 85, p. 312.

In the condition of local inflammation the heart beats forcibly, and the arteries conduct the full force of the wave to the swollen, tense, inflamed part; the part throbs with pain. A fomentation, by softening the arteries and the confining frameworks which surround the tissue cells, or the surgeon's knife by relieving the tension, permits an ampler flow of blood with its curative properties.

It is the altered osmotic condition of the infected inflamed tissue which causes the swelling, and this may advance to such a degree that the circulation is strangled and the part necrosed; before this happens, however, the full stroke of the heart's systole is conveyed by the hard contracted arteries with hammer-like strokes to the part and forces the blood through the vessels, maintaining the circulation and thus allowing the bacterial poisons to be neutralised up to the utmost possible limit. Probably the hypertrophy of the muscular coat of the arteries in certain pathological conditions is correlated with a need for the hammer-like stroke.

On the Non-identity of Trypanosoma brucei, *Plimmer and Bradford*, 1899, *with the Trypanosome of the Same Name from the Uganda Ox.*

By J. W. W. STEPHENS, M.D., D.P.H. (Cantab.), and B. BLACKLOCK, M.D., D.P.H.

(Communicated by Sir R. Ross, K.C.B., F.R.S. Received December 7, 1912,—Read January 23, 1913.)

Introduction.

Before considering our own observations it will be necessary to review briefly previous statements regarding the morphology of *Trypanosoma brucei.*

(1) Bruce (1) states that the hæmatozoa vary among themselves a good deal in shape and size and seem to take on slightly different forms in different species of animals. He publishes four figures depicting nine trypanosomes. Possibly one or two of the five figured from the dog might be considered to be "stumpy" forms.

(2) Kanthack, Durham, and Blandford (2) state that the Nagana parasites vary considerably both in size and form. They may be long and pointed and sometimes stouter, some individuals are short and thick with a short flagellum, their protoplasm being crowded with granules. This description suggests dimorphism, but it should be noted that forms without a free flagellum are not mentioned. No slides were available belonging to these observers, but Dr. Durham kindly lent us a large series of photographs. On examining these, one or, perhaps, two show a "stumpy" form, but it is difficult to be certain, and the uniformity of the remainder is striking. They state that "the material for our observation was obtained in the first instance from the blood of a dog infected by the disease on the voyage from Africa, and brought to England in November, 1896, by Dr. Waghorn."

This animal we believe to be the origin of the strain of *T. brucei,* Plimmer and Bradford, 1899, described by these authors, and at present maintained in England, so far as we can gather, solely at Liverpool.

It is not stated above from what animal the dog was infected on the voyage, nor is it stated what the exact original source of the strain derived from Zululand was.

(3) Plimmer and Bradford (3 and 4) describe four forms in the blood, but neither their description nor figures suggest that they have seen stumpy forms. They describe "a large hyaline form." "This organism is much larger than the ordinary adult form, and is much wider, often more than

double the width, and is more irregular in shape. The protoplasm is quite homogeneous and much more delicate, and it stains very faintly with the methylene blue." They are still to be found in films, and we easily found them in Dr. Plimmer's old films, but we found only extremely rarely forms that could be called "stumpy." We think that if they had been present these observers would hardly have failed to have noticed and drawn attention to them, as they have a striking appearance.

(4) Bruce and others (5) make a comparison between *T. brucei*, Uganda, 1909, and *T. brucei*, Zululand, 1894.

They state that many of the old Zululand preparations are still extant, so that it has been possible to do this. The preparations were, however, 15 years old, and had been stained with carbol fuchsin. The slides were got from horse, donkey, ox, monkey, dog; 200 trypanosomes were measured from the Zululand strain and 172 from the Uganda strain. The curves obtained in this way certainly resemble one another, though in one case the peak is at 18 μ, in the other case at 20 μ.

Also trypanosomes are figured from each strain, and there can be little doubt that there is a close resemblance, if not identity, viz., in the fact that both possess both long and stumpy forms. "With the evidence available the Commission consider themselves justified in considering the trypanosome recovered from the Uganda ox to be identical with *T. brucei*, the cause of Nagana in Zululand and other parts of South Africa."

Further, Bruce states in another paper (6) that *T. brucei* (Uganda strain) has actually 26 per cent. of non-flagellated forms.

(5) In 1911 Laveran (7) published an article, entitled "Identification et essai de classification des trypanosomes des mammifères."

In this article he places *T. brucei* in his Group I, "Trypanosomes chez lesquels le flagelle présente toujours une partie libre," whereas he places *T. gambiense* in his Group III, "Trypanosomes ayant des formes à flagelle libre et des formes sans flagelle libre."

Or, in other words, *T. brucei* is classed among the monomorphic trypanosomes while *T. gambiense* is among the dimorphic. We have then two opposite statements as to the morphology of *T. brucei*, (1) that it is monomorphic and (2) that it is dimorphic.

We possess two strains of (so-called) *T. brucei* in the laboratory, viz., the Zululand strain, of which we have given the origin above, and the Uganda strain from Surgeon-General Bruce, obtained originally from the ox in Uganda in 1909. These strains have been maintained continuously at Runcorn in a variety of animals, the Zululand strain for 4½ years, and the Uganda strain for 2¼ years. In the case of the Uganda strain it was lost in

1912 for a short period but was returned to us again by Prof. Mesnil, who had previously received it from us.

We made then a preliminary examination of these two strains, and found to our surprise that they could easily be distinguished morphologically.

We next proceeded to make a detailed examination of the two strains in a series of slides throughout the entire period of infection in various animals, viz., rats, guinea-pigs, and rabbits. As the result, we believe we have established the following facts :—

(*a*) The Zululand strain is *typically monomorphic.* The trypanosomes are long, with a long free flagellum. We must admit, however, that it is possible (as we believe is the case also in another typically monomorphic trypanosome, viz., *T. evansi*) to find by long search short forms which somewhat resemble true stumpy forms, but we must emphasise the fact that, in all the slides we have examined, prolonged search is necessary to find them.

(*b*) We have also verified the fact that Laveran's *T. brucei* strain also is, as he says, monomorphic. The origin of this strain seems uncertain. Laveran probably received it from Ehrlich, but where the latter got it from cannot now be ascertained. Unless it came from England, there must be two monomorphic *T. brucei* strains in existence, not to mention the possibility of other *T. brucei* strains of uncertain parentage in various laboratories.

We have examined also old slides from the Zululand strain lent us by Prof. Nuttall, Colonel Skinner, R.A.M.C., and Dr. Plimmer.* All these were monomorphic. We repeat here that in these films, or at least some of them, it was possible by long search to find a short form somewhat resembling a stumpy form, but not having the somewhat indefinable characteristic appearance of the latter.

(*c*) The Uganda strain, on the contrary, is *typically dimorphic, i.e.*, besides the usual long forms of trypanosomes, stumpy forms are readily found, even in abundance occasionally, when the infection is well marked. Bruce (6), as we have noted above, states that this trypanosome has 26 per cent. of non-flagellated forms.

The typical stumpy form we may define as a short, thick trypanosome, 12–14 μ, almost straight or slightly curved along one edge, while along the other the membrane is thrown into bold folds, there being no free flagellum, or at times a very short or doubtfully free one.

It is thus easy to distinguish a typical Uganda specimen from a Zululand specimen, and, in fact, we may express the difference in this way, that it is impossible to match a typical Uganda slide by any slide from the Zululand strain.

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* We desire here to express our thanks to these gentlemen for their kindness in sending us slides.

We have stated in the above history of the Uganda strain that it was recently returned to us by Prof. Mesnil, who remarked in his letter that he had maintained it in mice (for nearly a year), and that it showed now very few "trapues" forms. This we have been able to verify in the films made from the infected mouse sent to us. But, as soon as we had re-inoculated it into guinea-pigs, it again showed numerous stumpy forms. But the same does not hold good for the Zululand strain; in guinea-pigs, as in rats and rabbits, the strain is *typically monomorphic, i.e.* it does not show stumpy forms. We therefore conclude that the two strains, as we now possess them in the laboratory, are different.

How, then, are we to explain these facts? There seem to us three possibilities:—

1. That the strain we now possess, which we have been designating *T. brucei*, Zululand, is not this strain at all, but some other trypanosome inoculated erroneously during the course of inoculations extending over years. We think this view is untenable, for it would not explain the monomorphic character of the old slides we have examined, nor would it explain Laveran's monomorphic trypanosome.

2. While Bruce may have been working with a dimorphic trypanosome in Zululand, and still has slides showing these characters, it is quite possible that the strain sent by him to England was something quite different. This is all the more likely, as Bruce successfully infected dogs from a variety of wild game, viz., wildebeeste, kudu, bushbuck, and buffalo, and, as Bruce himself states, "when *T. brucei* was discovered in Zululand in 1894, it was naturally thought to be the one and only trypanosome in Africa," and no suspicion arose at that time of a multiplicity of trypanosomes in native game. This is the simplest explanation, and the fact that Plimmer and Bradford do not describe or figure stumpy forms, and our examination of Dr. Plimmer's slides had the same result, makes it probable that this is the true one.

3. That the strain originally sent to England was dimorphic, but that it has now become monomorphic. This may have come about in two ways:—

(*a*) The strain originally was a *mixture* of a long trypanosome and a stumpy trypanosome, and the stumpy has now died out. If this explanation were valid, it would probably imply that *T. gambiense* and other dimorphic trypanosomes were also mixtures. This we regard as a not impossible view, but one we cannot at present prove or disprove.

(*b*) The strain was originally *dimorphic* (but not a mixture), and that it has now become *monomorphic*. If this were so, it would modify materially our notions of specificity of trypanosomes, at least in laboratories. Of such a change we have at present not much evidence. We have noted, however, above that the Uganda strain kept in mice for a year was almost (but not entirely) monomorphic, but that in guinea-pigs it at once showed its normal characters.

It is impossible at present to decide between these explanations.

We come back, therefore, to the fact of which we ourselves have no doubt, viz., that the trypanosome that Plimmer and Bradford worked with, and which they named *T. brucei* in 1899, is certainly now a monomorphic trypanosome, and is not the same as the trypanosome from the ox described under the same name by Bruce and others in Uganda.

We believe, then, that the facts we have brought forward prove the non-identity of the Zululand and Uganda strains.

In order to avoid confusion, we think it advisable that this Uganda trypanosome should be re-named. We therefore propose for it the name *T. ugandæ*.

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The Action of Adrenin on Veins. (Preliminary Communication.)

By J. A. Gunn and F. B. Chavasse.

(Communicated by Prof. Francis Gotch, F.R.S. Received December 13, 1912,—
Read February 6, 1913.)

(From the Pharmacological Laboratory, Oxford.)

It would be remarkable if the vein wall were the only tissue in the body to possess contractile fibres without a functionally important duty of contracting. Very little attention, however, has been paid to physiological alterations in the calibre of the veins, though such alterations may be of high importance in modifying physiological and pathological conditions of the circulation, and in explaining certain actions of drugs.

The following investigation was undertaken in the hope of adding something to the knowledge of the contractile power of the veins; and, though the intended scope of the inquiry has not yet been completed, results have already been obtained which appear to be of sufficient importance to justify their being placed on record.

Method.

The method employed for recording the contractions of veins was similar, in essential respects, to that used by Cow* for determining the reactions of surviving arteries. In our experiments the veins were obtained from freshly killed sheep, and put, as soon as they could be obtained, into a Dewar flask containing oxygenated Ringer's solution at 37° C., and so conveyed to the laboratory.

A large water-bath, kept, unless otherwise stated, with a variation of half a degree on either side, at 36° C., held two beakers containing oxygenated Ringer's solution at the same temperature. In one of these beakers the veins were put until required; in the other was put the part of the vein used for each experiment. For these experiments ring preparations were made. It is difficult to cut quickly and without undue manipulation of the vein a ring of absolutely uniform cylindrical length; but the rings used had a length averaging 1·5 mm., which varied not more than $\frac{1}{2}$ mm. on either side at different parts of the ring. The ring was suspended between platinum hooks, the lower hook being fixed, the upper attached by a silk thread to a lever, which recorded variations in the calibre of the ring upon a slowly revolving drum.

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* Cow, 'Journ. Physiol.,' 1911, vol. 42, p. 125.

(a) *Veins Remote from the Heart.*

All the ring preparations of (large) veins which we have so far subjected to the action of adrenin have responded by contraction. In the experiments illustrated in the accompanying figures the magnification of movement was the same in each case. Fig. 1 shows the contraction of an external jugular, fig. 2 of a mesenteric, vein. In regard to the amount of contraction produced they are, however, not comparable, because the temperature in the case of the mesenteric vein was higher (41° C.) than in the other experiments, in which it was 36° C.

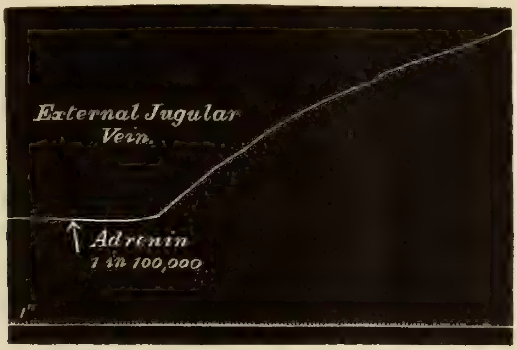

Fig. 1.

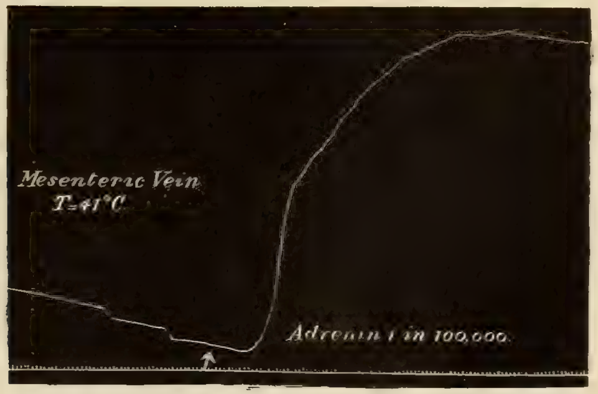

Fig. 2.

The fact that veins contract under the action of adrenin renders it highly probable that veins possess venoconstrictor fibres supplied from the thoracico-lumbar sympathetic system. Several observers have concluded that the portal veins contain venomotor nerves; but the presence of such nerves in other veins rests mainly on the evidence of Thompson* and Bancroft,† who found that stimulation of the sciatic nerve in the cat and rabbit produced visible contraction of the superficial veins of the hind limbs.

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* Thompson, 'Archiv f. Physiol.,' 1893, p. 102.

† Bancroft, 'Amer. Journ. Physiol.,' 1898, vol. 1, p. 477

(b) *Veins Near the Heart.*

Considerable interest attaches to the action of adrenin on the great veins near the heart. It has long been known that in the mammal the great veins near the heart, which correspond in some ways at least to the sinus venosus in the frog, beat rhythmically with the heart proper. One of us has on previous occasions made unsuccessful attempts to obtain a spontaneously contracting ring preparation of the superior vena cava of the cat and rabbit. A renewal of this endeavour with the larger rings from the sheep and bullock has been equally unsuccessful, though it is not suggested that this may not yet be accomplished.

What we believe to be a phenomenon of considerable interest and importance is that such a quiescent ring preparation of the superior vena cava near the heart can be made to beat vigorously and rhythmically by the action of adrenin.

For these experiments the venæ cavæ were removed along with part of the auricle, so that the distance of the ring from the auricle could be measured accurately.

Fig. 3 shows the effect of adrenin, 1 in 20,000, on a ring preparation of the superior vena cava of a sheep, 6 mm. distant from the angle of its junction with the auricle. The kind of effect produced by adrenin on this venous ring is exactly like that produced by it on the whole heart. Though it cannot be postulated with absolute certainty in the case of the quiescent tissue, inspection of the tracing leaves little room for doubt that adrenin augments and accelerates the contractions of the ring.

In the first place, therefore, this method of experiment affords evidence in favour of the view that the augmentor-accelerator nerve supply of the heart extends for some distance up the superior vena cava.

Secondly, it throws some light on the origin of the rhythmicity of the heart. The ring of the superior vena cava is quiescent. (This is established not only by the absence of movements of the lever, but also by observation of the ring in a strong light.) Adrenin almost immediately induces powerful rhythmic contractions. The action of adrenin is a continuous stimulus to which the muscle responds by a discontinuous (rhythmic) contraction.

Now the researches of Lewandowski, Langley, Elliott, Dale, and others have established with an unusual degree of certainty that adrenin acts on the myo-neural junctions of the thoracico-lumbar sympathetic system, and that its action is confined to these. Unless, therefore, the action of adrenin

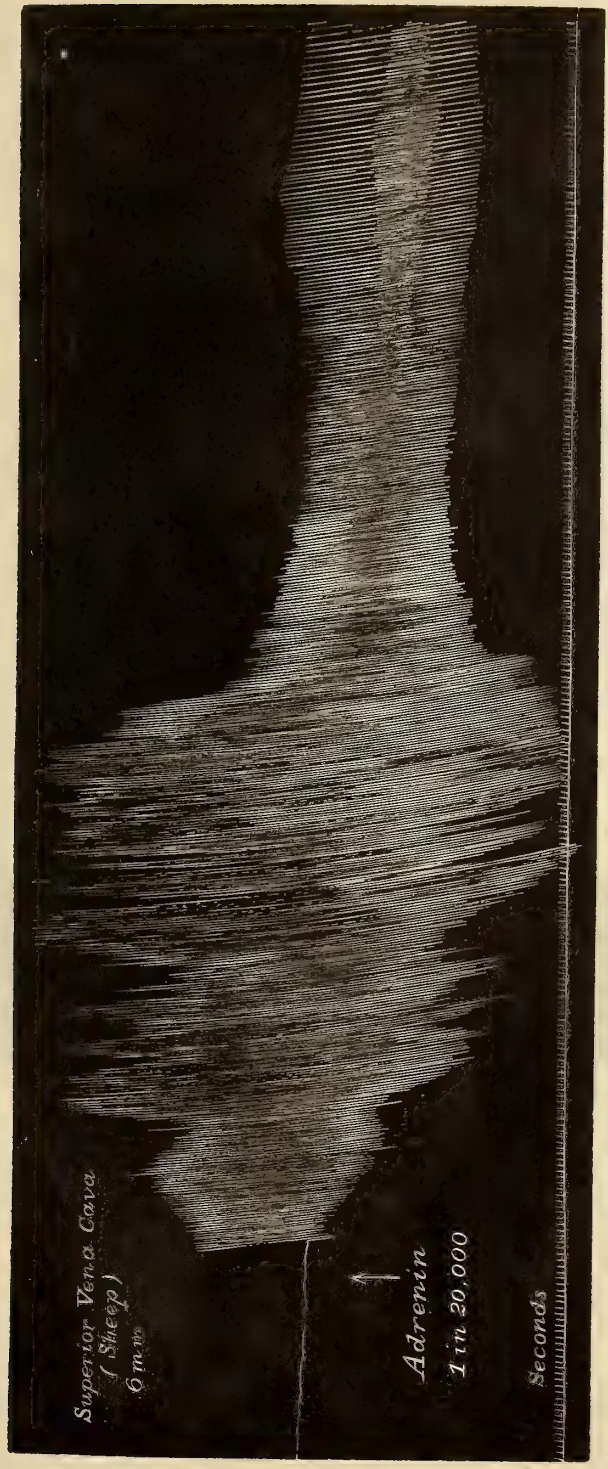

FIG. 3.

on the superior vena cava is unique, there are apparently only two ways in which its action on the quiescent superior vena cava can be explained.

On the neurogenic hypothesis of the rhythmicity of the heart, it is possible to hold that the continuous stimulation by adrenin of the sympathetic myo-neural junctions so raises the excitability of the muscle that previously subminimal rhythmic impulses from intrinsic motor ganglia (hypothetically present in the ring preparation) are now adequate to elicit rhythmic contractions.

On the myogenic hypothesis, on the other hand, stimulation of the myoneural junctions of the sympathetic nerve causes the muscle to enter into the rhythmic activity which is inherent in it.

All that can be said at present is that the latter explanation seems somewhat more probable. If, however, we should be able to elicit, by adrenin, rhythmic contractions in a ring of the superior vena cava in which subsequent histological investigation can reveal no ganglia, then it would furnish a cogent argument in favour of the myogenic hypothesis of the rhythmicity of the heart.

Further, this kind of investigation has afforded, and with further experiment, it is hoped will afford with greater accuracy, a physiological method of determining how far the rhythmically contractile tissue extends up the great veins, and where it merges into non-rhythmic contractile tissue. The difference in physiological reaction can be controlled by subsequent histological investigation. In the meantime it can be said that the rhythmically contractile tissue extends up the superior vena cava of the sheep for at least 6—8 mm. from the veno-auricular junction. Fig. 4 shows that 16 mm. from this junction, a ring of the same superior vena cava which gave the rhythmic contractions shown in fig. 3 responds to adrenin by simple contraction. It must be emphasised that a negative result in inducing rhythmic responses is not in itself conclusive, because, even at 6 mm. distance from the heart, adrenin does not always induce rhythmic contractions. Under the conditions of the present experiments it is not possible for the veins to reach the laboratory always in the same condition of excitability; and we have found

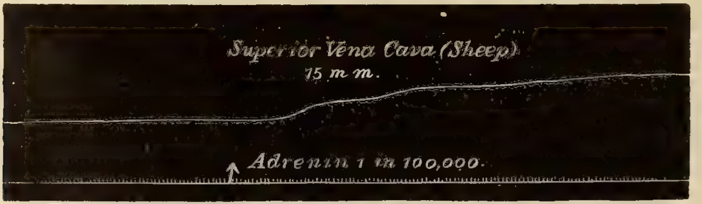

Fig. 4.

that a negative result is likely to arise when the muscle of the vein is subnormally excitable to electrical stimulation.

In the inferior vena cava we have on no occasion been able to induce rhythmic contractions. The rings of the inferior vena cava have always responded by simple non-rhythmic contraction, as shown in fig. 5.

These experiments are being continued, and extended to the action of other drugs.

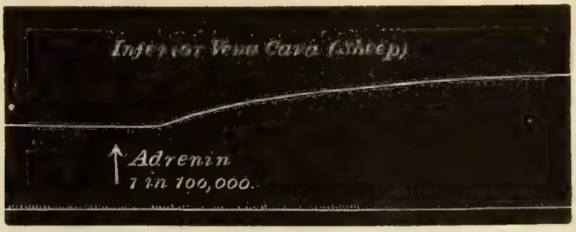

Fig. 5.

Summary.

1. The action of adrenin upon ring preparations of veins remote from the heart is to diminish their calibre, as in the case of arteries. They, therefore, probably contain venoconstrictor nerve fibres from the thoracico-lumbar sympathetic system.

2. The action of adrenin on quiescent rings from the superior vena cava near the heart is to cause them to beat rhythmically and powerfully.

3. (*a*) The accelerator-augmentor nerve supply of the heart, and (*b*) the rhythmically contractile tissue, extend up the superior vena cava for at least 6—8 mm. from the veno-auricular junction in the heart of the sheep.

4. The induction by adrenin of rhythmic contraction in the quiescent superior vena cava seems, on the whole, in accordance with the myogenic theory of mammalian heart rhythmicity.

An Apparatus for Liquid Measurement by Drops and Applications in Counting Bacteria and other Cells and in Serology, etc.

By R. Donald B.Sc. (N.Z.), D.P.H. (Oxf.).

(Communicated by Dr. L. Hill, F.R.S. Received November 21, 1912,—Read January 16, 1913.)

(From the London Hospita Bacteriological Laboratory. Dr. William Bulloch, Director.)

To promote drop-measuring in serological and bacteriological work, etc., the writer has devised a simple system of producing uniform pipettes, clean and sterile, which deliver uniform drops of any required size from ¼ c.c. down to 1/200 c.c. or less, and has devised also simple forms of constant-pressure apparatus for use with the pipettes.

The fundamental principle of his method rests on the fact that the size of a drop of a given liquid yielded by a clean pipette is determined by the outer circumference of the pipette at the level where the contact-edge of the drop clings round the glass—due allowance being made for the rate at which the drop is detached and the temperature.

The pipettes, freshly drawn out from glass tubing in a Bunsen flame to a nearly cylindrical capillary form, are gauged in a wire gauge and cut off at the required sizes. The gauges used are such as the Starrett Morse Drill and Wire Gauge, which has holes ranging in diameter from 5·79 mm. down to 0·34 mm.

Tubes larger than these sizes may conveniently be gauged by the Columbia vernier slide gauge. Capillary tubes less than 0·34 mm. may be gauged in a wire drawplate.

In gauging, the capillary tube is pushed gently down into the particular gauge hole required and is then cut off—preferably at the upper surface of the plate, so that the dropping-point shall not come into contact with any trace of greasy matter which may remain on the cleaned gauge plate.

To ascertain the size of drop yielded by such a dropping-point an adjustable constant-pressure apparatus was devised. This (fig. 1) consists of a straight tube of 3 to 4 mm. internal diameter and of such a length, *e.g.* 50 or 60 cm., that the free air space within shall be amply greater than the capacity of the pipette employed. The tube is carefully cleaned, washed finally with distilled water, and dried with grease-free cotton wool drawn through on a thread. The ends are opened out slightly funnel-shaped to facilitate the ramming in of an inch or so of pure cotton wool, which is required to retard the passage of air and to prevent effectively the escape of any of a column of pure dry mercury, 20 cm. long, which is introduced to act as a plunger.

The tube is arranged as shown in the figure. The right-hand end is joined by a short rubber tube to the upper end of a pipette, which is supported by a clamp, and the left-hand end, attached to a screw-clamp, can be moved up or down the tall stem of a retort stand.

The stem of the pipette has an internal diameter of 1 mm. or so, and the lower of its two calibration marks is 1 or 2 cm. below the bulb. Then 1 or 2 cm. below this the pipette is joined by a short piece of cleaned bicycle valve-tubing to the upper end of the capillary dropping nozzle. For special work the junction may be made by means of a sleeve of good cork or by grinding or fusing the nozzle on to the pipette point.

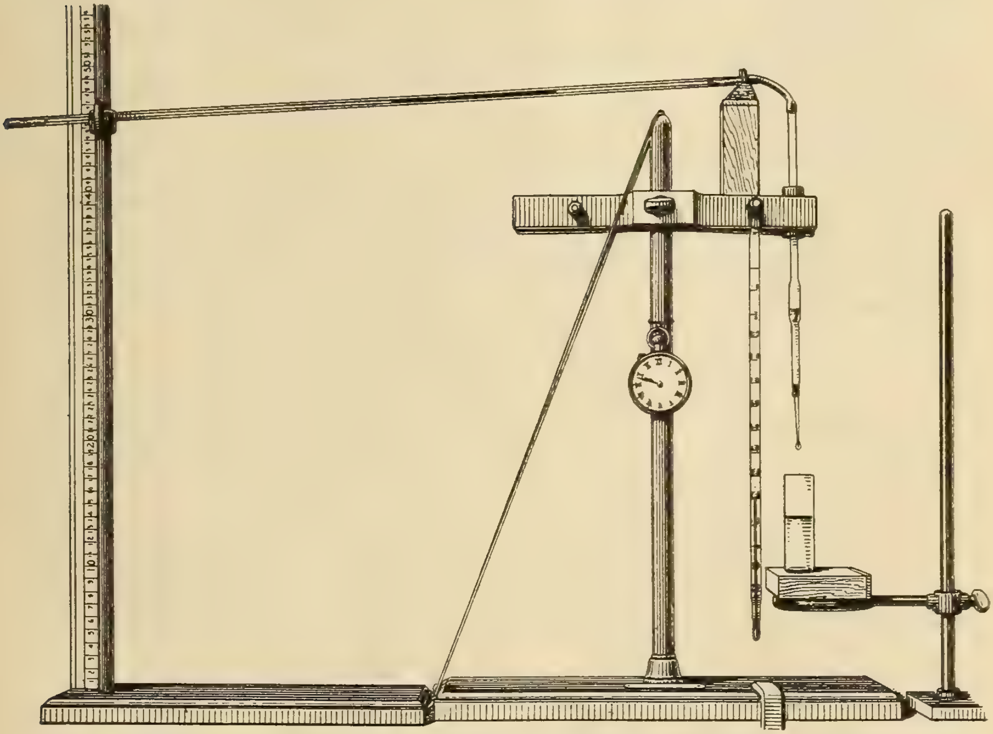

Fig. 1.

The liquid friction in a small dropping nozzle ought to be such that the head of mercury employed may be great enough to render negligible the loss of 2 or 3 cm. head of water as the pipette empties. The acceleration of the falling mercury down the well-throttled sloped tube is negligible.

To carry out a dropping experiment the mercury is first brought to the right-hand end of the tube, or, if the dropping nozzle has great friction, to within 1 or 2 cm. of the end. The vessel of liquid is raised to cover the end of the nozzle. The left-hand end of the mercury tube is then depressed till the pipette is nearly full. Next it is gently raised until the upper meniscus of the liquid rests at the upper calibration mark. Finally the vessel of liquid is lowered.

Now the end of the mercury tube is raised till the drops fall at the required standard rate, say one per second. The height required for the dropping-point in use is marked by a sliding ring. Then the pipette is refilled, the mercury tube is at once elevated to the required point, and the drop-count at the uniform standard rate is observed.

If necessary, the mercury tube may be slightly lowered at the last drop to allow estimation of the fraction of a drop.

The drop-counts for distilled water thus found are given in the following table. The fourth column contains the quotient drop-weight in mgrm./diam. in mm., *e.g.* for Morse gauge 80, 1000/131/0·340 = 22·45. These quotients are seen to form a fairly uniformly falling curve as the dropping-point increases in size.

Drop-count. Distilled Water.

Morse gauge No.	Diameter.	Drop-count (20° C.) per 1 c.c.	$\frac{\text{Wt. in mgrm.}}{\text{Diam. in mm.}}$
	mm.		
80	0·340	131·0	22·45
79	0·366	122·0	22·4
78	0·406	112·9	21·9
77	0·457	101·0	21·74
75	0·533	87·0	21·6
72	0·633	73·5	21·4
68	0·787	58·5	21·4
66	0·838	56·5	21·1
62	0·965	51·6	20·1
60	1·016	49·8	19·9
58	1·067	48·3	19·53
57	1·092	47·9	19·1
54	1·397	38·9	18·4
52	1·612	34·0	18·25
43	2·261	25·5	17·4
33	2·870	20·3	17·2
28	3·569	16·7	16·8

At different rates of dropping the drop-count differs as shown by the following observations:—

	Diameter.	Sec. per drop.	No. of drops obtained.
	mm.		
Dropping-tube (throttled on separator cylinder with Mariotte's tube)	14·4	0·5	41·0 from 10 c.c.
		0·6	41·5
		1·0	42·0
		2·73	46·0
Dropping-tube similarly fitted	8·4	0·5	76·0
		2·5	84·0
Mercury tube pressure, Morse 33.........	2·87	0·5	22·2 from 1·125 c.c.
		1·0	23·0
		2·7	23·2
		3·0	23·9
		4·3	24·3
		12·0	24·6
,, ,, Morse 56.........	1·181	0·27	45·0
		0·44	46·0
		1·0	50·0
		1·6	51·3
		3·0	51·5
,, ,, Morse 80.........	0·34	0·5	68·0 from 0·5 c.c.
		0·75	67·0
		1·0	66·0
		2·0	67·0
,, ,, D.P. VIII	0·29	0·32	95·0
		0·6	90·0
		1·0	86·4
		1·33	86·1
		1·63	86·2
		3·0	86·4
Hand-teat pressure, D.P. X	0·25	0·43	116·0
		0·7	106·0
		1·0	99·0

From the above observations it may be seen that just in the cases where fine accuracy is most desirable—namely, in measuring by hand one or two drops—these small drops have in their favour the peculiarity (pointed out by Ollivier) of being practically constant in weight at all expulsion-rates slower than one drop per second. Such rates can be easily and reliably secured by the use of a hand mercury-plunger tube—a miniature form of the mercury tube described above.

The narrow limits of drop-rate required for large drops, *e.g.* of 1/5 to 1/4 c.c. in the Wassermann test, may be secured by the use of the arrangement shown in fig. 2.

A separate cylinder of convenient size has a small Mariotte's tube fitted by a rubber cork through the upper aperture. Various dropping-tubes, smaller to telescope inside, or larger to telescope outside the stopcocked tube, may be fitted on by a ferrule of washed rubber tubing or of good cork. A dropping-point to give watery drops of $\frac{1}{4}$ c.c. may be formed of tubing $14\frac{1}{2}$ mm. external diameter. The dropper is held in a stand at a height convenient to allow a rack of test-tubes to be slid under the dropping-point.

The drop-rate may be regulated as desired by fitting inside the dropping-tube, and within forceps-reach of the orifice, a throttle made of suitably drawn out capillary tube.

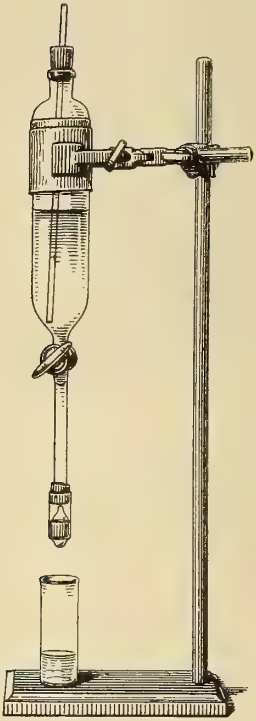

Fig. 2.

The author has found the apparatus efficient in the following applications:—

(1) Comparison of surface-tension of liquids, in quantity as little as $\frac{1}{2}$ c.c., *e.g.* in testing the value of the meiostagmin reaction in syphilis.

(2) As the drop yielded by one pipette differs by not more than a fraction of 1 per cent. from the drop similarly yielded by another pipette similarly gauged in the same hole, the method may be used, with successive fresh pipettes, for rapidly and accurately measuring off small quantities of any liquid, from 0·25 c.c. to, say, 0·004 c.c. of watery liquids of high surface tension, or even smaller quantities of liquids of low surface tension, for instance, in:—

(*a*) Measurements of various small quantities, in *e.g.* Wassermann reaction, micro-Wassermann reaction, and other complement-fixation tests; Widal reaction; alkalimetry, acidimetry, and other volumetric tests; testing cultures and disinfectants; and in pharmacy.

(*b*) Direct estimation of cells, numerically, and roughly qualitatively, in small drops of cerebro-spinal fluid, dried on a slide.

(*c*) Blood-count, red cells and white cells, from drops of diluted blood, similarly dried.

(*d*) Direct counting of bacteria in small dried drops of diluted vaccines, distilled water, domestic water, sewage effluent, diluted milk.

(*b*), (*c*), and (*d*) yield permanent preparations.

A Preliminary Report on the Treatment of Human Trypanosomiasis and Yaws with Metallic Antimony.

By H. S. Ranken, M.B. Glasg., M.R.C.P. Lond., Captain R.A.M.C., Member Sudan Sleeping Sickness Commission.

(Communicated by H. G. Plimmer, F.R.S. Received December 23, 1912,—Read February 6, 1913.)

(From the Yei Camp, Sudan Sleeping Sickness Commission.)

The use of precipitated metallic antimony, in a state of finest division, was devised by Plimmer for the treatment of trypanosomiasis, and the results of his and other experiments with this substance have been published.* After a long series of experiments on subcutaneous and intramuscular injections of this form of antimony suspended in water, oily media, egg yolk, etc., all of which caused great irritation, he found that it was possible to inject it intravenously with safety, and without causing any irritation of the tissues. A large number of animals were cured of trypanosomiasis by this means, and in May, 1910, Major W. B. Fry gave a dose intravenously to a late case of Kala Azar, thus demonstrating that it could also be used safely in this manner on human beings.

Captain R. J. C. Thompson, R.A.M.C., gave this preparation by intravenous injection to the cases first admitted to the Yei Sleeping Sickness Camp between January and March, 1911. Eighty-one injections were given to 38 cases, but pressure of administrative work prevented these cases being fully treated and investigated, and they were all transferred to atoxyl treatment. Captain Thompson states that from a clinical standpoint some of these cases showed great improvement.

Later I had the honour of being appointed to the Sudan Sleeping Sickness Commission for work at Yei in the Lado Enclave, and since October, 1911, I have collected a series of cases treated by metallic antimony alone and in conjunction with other drugs. This report is consequently only preliminary; but it demonstrates that antimony in this form is a safe drug to employ in the treatment of sleeping sickness, if used with reasonable precautions, and that the results obtained so far certainly call for extended use and further investigation.

During the past year 76 newly admitted cases have been treated with antimony, alone and combined with salvarsan and atoxyl, and shorter courses of antimony have been given to 143 old cases previously treated with atoxyl, atoxylate of mercury, etc. Over 1400 intravenous injections have been given, and three deaths have occurred which can be attributed to the treatment. All these fatal cases were amongst the first 150 injections.

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* 'Roy. Soc. Proc.,' B, vol. 80, p. 483; B, vol. 81, p. 354; and B, vol. 83, p. 140.

The method of administration is the same as that of an ordinary intravenous injection of saline solution given hydrostatically through a needle of somewhat large bore.*

The dose of antimony is stirred with about half an ounce of normal saline in a small glass mortar and becomes a temporary suspension. Two ounces of saline are then poured into the funnel and tubing, the needle is inserted into any vein in the forearm and the clip opened. As soon as it is seen that the saline is flowing freely into the vein, and there is no swelling round the site of the puncture, the suspension of antimony is poured into the funnel and the mortar is washed out, with a little more saline, into the funnel in order to leave no residue. The antimony is allowed to run into the vein, the funnel being gently shaken from time to time, and, when it is on the point of becoming empty, more saline is poured in. The window in the rubber tubing should be watched, and after all traces of antimony have passed it some more saline is allowed to run in to clear it out of the part of the tubing below the window; the clip is then closed and the needle withdrawn. About six ounces of saline seems to be a sufficient quantity, and the time occupied in giving an injection varies from three to seven minutes—depending on the calibre of the vein and the bore of the needle employed.

I have found it possible, without any hurry, to give as many as 10 injections in an hour; I mention this to show that this method of treatment is feasible on a large scale.

The question of dosage is one of considerable importance. The dose usually given was one grain. Large doses up to three grains have been given, but these seem to be attended with risk and have been given up for the present; it is probable, however, that in good, selected cases one and a half, or even two, grains may be safely given. Several cases of extreme susceptibility have been met with, however, so that the initial dose should be one grain. The interval between the doses seems to be of the greatest importance. Four days would seem to be most suitable, but injections have frequently been repeated on the third day after a dose; they should certainly not be postponed any later than the fifth day. A few of the earlier cases were treated with weekly doses; two of these were very susceptible and were unable to take doses at shorter intervals; both cases have relapsed.

The usual course of treatment has been five doses at intervals of four days, covering a period of from 17 to 20 days; after an interval of six weeks a short course of three doses extending over nine days has been given.

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* The size of the one I have used is No. 19 of Imperial Standard Wire Gauge internal diameter and No. 15 external.

The table on pp. 206–7 gives in shortest form the results of the treatment with antimony alone. Thirty-five cases have been so treated: in 15 of these there were no symptoms other than enlargement of the cervical glands, except varying degrees of debility; 17 had also fine tremor of the tongue and five œdema of eyelids. The cases were not especially selected for treatment, but were taken just as they came.

Up to the present it has not been possible to procure susceptible animals for inoculation from the cases treated, so microscopical examination has been the only available method of controlling the results. Blood and gland juice have been examined in all cases. Three observers have undertaken each examination, and the total time of each examination has been one and a half hours. Many of the cases have been repeatedly examined.

Of these 35 cases four are dead and two have deserted. Trypanosomes have reappeared in the blood of four cases and another has relapsed clinically. The remaining 24 are all improved and all microscopical examinations are negative up to date of writing.

(a) *Deaths.*—Two only of the four deaths can be connected directly with the treatment. Both were advanced cases and had been given larger doses than usual. No. 2, a boy of 10 years, had 2 gr. on one occasion and 1½ gr. twice. He showed no symptoms till 48 hours after the last dose, when meteorism appeared accompanied by acute abdominal pain, and he died seven hours later. I have seen a somewhat similar death here in a case treated only with atoxyl.

No. 24 was also an advanced case; he took two injections of 1 gr. without discomfort and then had a dose of 2 gr. Twelve hours later epileptiform symptoms set in quite suddenly; the patient became unconscious and died six hours later.

Of the other two deaths—No. 7 was due to broncho-pneumonia; the patient was debilitated and broncho-pneumonia was very prevalent at that time. No. 47 died from an epileptiform attack, but had not had any form of treatment for a week.

(b) *Deserters.*—One, No. 38, had recently completed his course of treatment and had shown great improvement. The other, No. 3, belonged to a district 100 miles from Yei. He had been without treatment for five months and had done very well—taking large doses without untoward symptoms. All examinations had been negative, though repeated very frequently.

(c) *Relapses.*—No. 27 is a clinical relapse. He was a soldier and his

Abstract of Cases Treated with Antimony only.*

No.	Sex.	Age.	Glands.	Trypanosomes.	Treatment.	Present Condition.			General Condition.
						Blood and gland juice.	Glands.	Mental.	
1	F.	Adult	+ +	+ + +	5 doses 3 ,,	–	Much smaller. Puncture negative	Much improved	Much improved; better nourished.
2	M.	10	+ + +	+ + +	3 ,, (2 of 1½ gr., and 1 of 2 gr.)	–			Died 13 days after commencement of treatment; a very advanced case.
3	M.	Adult	+	+	4 doses (1 to 3 gr.) 3 ,, (1 gr.)	–	Disappeared	Good	Deserted, as he lived 100 miles away.
4	M.	,,	+ +	+ +	4 ,, (3 gr.) 3 ,,	–	Just puncturable	,,	Very much improved.
5	F.	,,	+	+	3 ,,	–	Disappeared	,,	In very good condition. Has recently been confined of a living child, and is quite well.
6	M.	,,	+	+ +	5 ,, (1 gr.) 3 ,, 3 ,, after relapse				Very susceptible to antimony. Remained in good condition for 5½ months, when he had a relapse.
7	M.	,,	+ +	+ + +	4 ,, (1 gr.)	–			Old and debilitated; died of broncho-pneumonia, which was prevalent.
9	F.	,,	+ +	+ +	5 ,, 3 ,,	–	Disappeared	Good	Very good.
13	M.	,,	+	+	5 ,, 3 ,,	–	,,	,,	Very good.
14	F.	,,	+ + +	+ + +	5 ,, 3 ,,	–	Scarcely noticeable	,,	Old; poor condition.
21	M.	,,	+ + +	+ +	5 ,, 3 ,,	–	Small and hard	Very good	Had œdema, which disappeared.
22	M.	,,	+ + +	+ + +	5 ,, 3 ,,	–	Much reduced	Good	Very good. Had tongue tremor, which disappeared.
24	M.	,,	+ +	+ + +	3 ,, (1 to 2 gr.)				Advanced case, in poor condition. Died with epileptiform seizure about 12 hours after 2-gr. dose.
25	M.	,,	+ +	+ +	5 ,, 3 ,,	–	Only one small hard one left	Good	Very good. Tongue tremor disappeared.
26	F.	10	+ +	+ +	3 doses (½ gr.) 3 ,, 3 ,,	–	Much smaller	Much brighter	Improved.
27	M.	Adult	+ + +	+ + +	5 ,, 3 ,,	–	Much less		This case was verging on the third stage; for 3½ months there was great improvement, so that he could act as assistant. He has now œdema, and is "mental." Blood, glands, and cerebro-spinal fluid are negative.

28	M.	11	+ +	+	5 „ 3 „	(¾ gr.)	—	Small and hard	Very good	Was in poor condition, with œdema.
29	M.	Adult	+ + +	+	3 „ 3 „ 3 „	....	—	Nearly gone...	Good	Very good. Had bronchitis after first course.
30	M.	„	+ +	+	3 „ 3 „ 3 „	....	—	„ „	„	Very good.
31	M.	„	+ + +	+ + +	5† „ 3 „	....				Had relapse two months after last dose. Trypanosomes appeared again in blood. Very sensitive to antimony.
33	M.	„	+ +	+ +	5 doses 3 „	....	—	Only one perceptible	Good	Came in bad condition, and showed great improvement. Œdema and tongue tremor disappeared.
34	F.	„	+	+	5 „ 3 „	....	—	Quite soft ...	„	Much improved.
35	F.	„	+ +	+ +	5 „ 3 „	....	—	Not noticeable	„	Very good.
36	M.	„	+	+	5 „ 3 „	....	—	Much smaller	„	Improved.
37	M.	„	+ +	+ + +	5 „ 3 „	....				Trypanosomes reappeared after two months.
38	M.	„	+ +	+ +	5 „ 3 „	....	—			Patient deserted six weeks after course of treatment in much improved condition.
40	F.	„	+	+	5 „ 3 „	....	—	Very small ...	Good	Very good.
41	F.	„	+ +	+	5 „ 3 „	....	—	Much smaller	„	Improved.
42	F.	„	+ +	+ +	5 „ 3 „	....				Trypanosomes reappeared after 2½ months.
45	M.	„	+ +	+	5 „ 3 „	....	—	Much smaller	Good	Good.
46	M.	„	+	+ +	5 „ 3 „	....	—	„ „	„	Came very thin; now much improved in condition.
47	M.	„	+	+	3 „	....	—			An old emaciated man, in advanced stage. Died a week after last dose in an epileptiform attack, not apparently connected with the treatment.
48	M.	„	+ +	+ +	5 „ 3 „	....	—	Much smaller	Better	Had œdema, which disappeared. Slight improvement.
50	M.	„	+ +	+ +	5 „ 3 „	....	—	Small	Good	Good.
51	M.	„	+	+ +	5 „ 3 „	....	—	„	„	Toluidin blue, 1 gr., was given before first, third and fifth doses at suggestion of Dr. Balfour. Tongue tremor disappeared; much improved.

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* Too much importance should not be attached to the size of the glands or number of trypanosomes, as these factors do not give an accurate indication of the severity of the case.

† At intervals of one week.

condition on arrival at the Camp was alarming, and he was quite unable to walk. A full course of antimony, in doses of 1 gr., was successfully given and there was a great improvement in his condition. The large masses of glands were very greatly reduced in size. The tremor was unaffected, but he became much stronger. He was able to act as a "native assistant" in the laboratory and a "headman" in the camp. His improvement was maintained for three and a-half months, when suddenly he relapsed and became a third stage case. Blood examinations have been negative. The cerebro-spinal fluid was examined also and the cellular elements were increased, but trypanosomes were not found.

In four other cases trypanosomes have reappeared in the blood. Two of these were very susceptible to antimony and were unable to take doses at shorter intervals than a week. After the relapse attempts were made to give them another course of antimony, but it had to be given up owing to their unusual susceptibility to the drug. The other two had heavy infections, but did not seem to improve. They have all been transferred to atoxyl.

(d) The other 24 cases have shown improvement. In three it is slight and these are being kept under close observation. In some cases the improvement has been very striking; in others who were admitted in fairly good condition, it has not been so evident, but the whole appearance of the patients is different. After a course of antimony there is not uncommonly depression and debility. This, however, passes off in a few days; the patient becomes more active, feels and looks better, and loses the languor that is so often seen in patients just admitted. They put on weight and the skin becomes healthier; this is no doubt due in part to the regular feeding in the sleeping sickness camp, but the improvement would not occur were it not initiated by the treatment. In some cases the tongue tremor has disappeared under treatment. Probably some other cases will relapse, so these cases are being kept without further treatment at present, as it is desirable to test the effect of this treatment with antimony alone.

II. *Antimony and Salvarsan.*

Ten cases have been treated with these two drugs, but a regular course has not been carried out; the salvarsan was given, in the majority of cases, when a patient was unable to take a complete course of antimony.

No information has been obtained as to the best line of treatment to be followed with this combination of drugs, but a further series has been commenced.

The results up to the present are: Eight out of the ten cases are very well and have shown definite improvement; one deserted, but was in very good health and had been without treatment for four months. She was one of the few who had a complete course. The tenth case had one injection of salvarsan and was unable to take antimony on account of extreme susceptibility. Atoxyl was substituted, but the patient developed toxic symptoms. She is now having small doses of atoxyl, but is getting steadily worse.

The salvarsan has been given both as an intramuscular injection in olive oil, and by intravenous injection in alkaline solution.

III. *Antimony and Atoxyl.*

The patients in this series have been treated as follows :—

(1) Five doses of antimony 1 gr. at intervals of four days.
(2) Atoxyl 5 gr. every three days for 40 days.
(3) Three doses of antimony 1 gr. at intervals of four days.
(4) Atoxyl 5 gr. every three days for one month.

There is no interval between these courses, so the patients have continuous treatment over a period of rather more than three months. An interval of a month is then given and atoxyl treatment is then continued, as a tonic and precautionary treatment.

Thirty-one patients have been treated; but three were unable to take the complete course of antimony.

This is the most recent series, and the three months' course has just been completed; it is too early to speak of results. At the time of writing all have done well; the improvement, in many instances, has been striking; some, however, were advanced cases, and it is not expected that the improvement can be maintained permanently.

IV. *Old Atoxyl Cases, Treated with Short Courses of Antimony.*

After we had seen that intravenous injection of antimony was bringing about improvement in the newly admitted cases and could easily be carried out on a large scale, it was decided to give short courses of antimony to the old cases that had been under treatment with atoxyl, atoxylate of mercury, etc. The majority of the patients so treated have had two separate courses of three doses of antimony, 1 gr.

In some instances, patients have not had all these six doses on account of susceptibility, intercurrent affections, bronchitis, etc., but 813 injections have been given to 143 patients.

In this series there has been one death. The two fatal cases in the first series had doses which have been found to be inadvisable in advanced cases. In this case only two injections of 1 gr. had been given. The patient was a man in good health; he showed slight depression after the first dose, but had quite recovered and was ready for a second dose four days later. He was sick after this and soon developed alarming symptoms, and died. There were no late effects in any of the other patients.

SYMPTOMS FOLLOWING THE INTRAVENOUS INJECTION OF ANTIMONY.

Antimony has a powerful depressant action and, as the preparation employed is very active, it is only to be expected that a course of treatment should cause the appearance of some symptoms. It has been found that some patients have a high degree of susceptibility to antimony, and they have suffered somewhat severely; also, at the beginning of this work, when attempts were being made to obtain some information as to the dosage, larger doses were given and the effects were, in some cases, more marked. With further experience symptoms of any degree of severity have become very rare.

The following are the symptoms which may be seen in patients who are not unusually susceptible:—

1. Fever: The "réaction thermique."

2. Pulse: Is not much accelerated; but, so far as can be ascertained by digital examination, there is a fall in blood pressure.

3. Diuresis: There is variable diuresis.

4. Cough: This occurs in the majority of cases. It begins a few minutes after administration and lasts for 5 or 10 minutes. Very rarely it has persisted for 12 or 24 hours, but only in cases which have had slight bronchitis.

5. Pain in the xiphisternal region: This is a common occurrence and is most severe the day following an injection. It is probably of gastric origin.

6. Headache is not uncommon, but is not severe.

The following symptoms have only occurred in cases of great susceptibility to the drug:—

7. Sickness and vomiting: This has occurred six times; once it occurred in a fatal case (No. 32A).

8. Fainting: Only one case.

9. Meteorism: Was seen in one case (No. 2), which was fatal. Captain Thompson saw a similar case 18 months ago.

10. Herpes: There have been seven cases. In six it was seen on the face and lips, and in the seventh along the ribs. This condition is probably similar to the herpes that occurs in certain forms of arsenical poisoning.

11. Stomatitis : Two cases; one was vesicular, but the other was more severe and ulcerated. In both cases the lesions were limited to the anterior portion of the hard palate, and the condition may perhaps have been herpetic.

It is of interest to note that very similar symptoms were observed in experimental dogs treated in this manner.

Short mention must be made of the effect of antimony on the temperature and upon the leucocytes.

I. *Temperature.*

A "réaction thermique" has been described, occurring about 20 minutes after injection of salts of antimony. This subject may be of greater interest owing to the large amount of work done on the subject of salvarsan fever.

Temperatures have been taken in over 100 cases: (1) at short intervals on the day of an injection ; and (2) morning and evening temperatures for three days after treatment.

There is no effect on the temperature till two or three hours after treatment, when it rises 1° F., and at four hours after treatment the average rise is 1·4° F. The same evening, 10 to 12 hours after treatment, some cases have fallen to normal, while others have gone up to over 101°. For the next two days there is an average evening temperature of 100·2° and 99·7°, and the temperature returns to normal. The results are identical in treated and untreated cases ; trypanolysis and the disposal of dead trypanosomes do not, therefore, cause this rise of temperature.

The initial rise may be due to antimony, but the temperature for the two following days is probably due to the saline solution. With the apparatus available here it is impossible to get pure distilled water; it would be of interest if some injections could be given with water twice distilled in a good apparatus.

The temperature does not seem to have any effect on the general condition of the patients.

II. *Leucocytes.*

The action of leucocytes on this preparation has been described.* Blood films, taken from animals after treatment, were stained and the leucocytes found to be crammed with the minute particles of antimony. They show a great avidity for this preparation, which they discharge, presumably in soluble form, into the blood-stream, the action being thus spread over a longer period.

A series of 200 leucocyte counts has been made to determine the effect of treatment on the numbers of leucocytes. The cases selected were all under treatment, but some enumerations were made on untreated cases and the same changes observed.

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* 'Roy. Soc. Proc.,' B, vol. 83, p. 140.

Immediately after injection of antimony there is a considerable reduction of the leucocytes in the peripheral blood and in half an hour they have fallen to 60 per cent. of the number obtained on enumeration just before administration. In some cases the count is still lower an hour after the injection. From this point the leucocytes began to rise in number and in the majority of cases have returned in four or six hours to the level of the first count. This increase continues and 24 hours after treatment there is an average count of over 16,000; this is maintained for another 24 hours, but four days after treatment the numbers have fallen to approximately the original count. The estimations were continued over a series of three doses, and showed the same changes after each.

III. *Action of Antimony on Trypanosomes.*

The exceedingly rapid action of the salts of antimony in man has been reported (4), but it was not anticipated that the metal would have an almost immediate trypanocidal action. Trypanosomes were never found in cases examined a few hours after treatment, so observations were made to determine the time required for an intravenous injection of antimony to clear the glands of trypanosomes. Only heavily infected cases were selected, a case being considered suitable if 10 trypanosomes were found in five minutes in the gland juice, but in many instances they were much more numerous, two and three trypanosomes being often seen in one field. The time was taken from the moment the antimony suspended in saline solution entered a vein in the arm, and at periods from 3 to 30 minutes after this time glands were punctured and wet preparations made. These were examined both by dark ground and ordinary illumination. In all cases 15 minutes was allowed for each film, and in many cases the search was prolonged up to 30 or 45 minutes.

After 3 minutes (slide made before the injection is completed).—Trypanosomes are still abundant, but some are already very obviously affected by the treatment. They show greatly exaggerated activity and dash rapidly across the field, so that they are difficult to keep in view. Frequently a trypanosome becomes anchored by the blunt end and lashes about most vigorously. This exaggerated motility soon passes into a state of fatigue, when the trypanosome wriggles more slowly and more and more feebly till movement ceases. Some retain their normal form, while others become swollen and bloated.

Occasionally a trypanosome comes rapidly to a standstill and dissolves away so that only a haze of protoplasm can be seen left behind.

After 5 *minutes.*—The exaggerated motility is not so frequently observed, but the trypanosomes are more often anchored and may be seen lashing about in an extreme state of activity. They appear to be somewhat reduced in number.

After 7 *minutes.*—Trypanosomes are more scanty. Exaggerated motility is not a feature of these preparations, but all the other changes occur as in the earlier preparations.

After 10 *and* 15 *minutes.*—In all preparations taken at these times a considerable search was required to find a trypanosome, but in the majority of cases one could be found in a search of 10 to 20 minutes.

After 20 *minutes.*—Very many preparations have been examined and have invariably proved negative.

An intravenous injection of antimony therefore kills the trypanosomes in the circulating blood in 20 minutes.

On the Treatment of Yaws by the same Method.

Some cases of yaws have been met with in the course of this work.

In view of the successful results published by Strong, Castellani, and Alston, the first three cases were treated with salvarsan. One was given an intramuscular injection of 0·6 grm. in olive oil; the lesions—plantar ulcers—were healed in three weeks. The two others had an intravenous injection of 0·45 grm., they showed several small patches on the face and trunk and responded much more rapidly to intravenous treatment.

I had been much impressed with the rapid trypanocidal action of this metallic antimony, and decided to treat a case of yaws with this drug. At first doses of 1 gr. were given and the condition improved, but not very rapidly. With a larger dose the effect was much more striking; 1½ gr. seems to be quite efficient, but 2 gr. have been given to the last four cases. None of the 10 cases manifested the hyper-susceptibility to antimony that has been seen in sleeping sickness, and there have been no after effects.

In an adult of fair condition 1 gr. should be given as a first dose, and doses of 1½ gr. or 2 gr. repeated twice with intervals of four days. I have sometimes shortened the interval by one day. Three doses, I believe, is a quite sufficient course of treatment, but in the majority of our cases a fourth dose has been given to ensure, as far as possible, a permanent result, as most of these cases have been collected from different villages and pass out of observation when they are discharged from hospital. The antimony is administered as described above (p. 204) for trypanosomiasis.

The following short notes give an abstract of the cases:—

1. Lesions small, but generally distributed with septic or impetiginous patches in the beard. Four doses of antimony were given—two of 1 gr. and two of 1½ gr. Lesions were all healed in 12 days.

2. Lesions general and larger than in preceding case. Treatment, four doses of 1½ gr. All lesions healed in 11 days.

3. Extensive ulceration of scrotum. The ulcers had moist surfaces and an exceedingly offensive discharge. Treatment, four doses of 1½ gr. All healed in 11 days.

4. A large crusted patch on perineum and several small lesions on face. Treatment in four doses of 1½ gr. Healed in 10 days.

5. Extensive ulceration on soles of feet. Treatment four doses of 1½ gr.; local treatment, perchloride of mercury 1/1000 as a lotion. Quite healed in 14 days.

6. Discharging ulcers between toes. Treatment, four doses of 1½ gr. Healed in 14 days.

7. Generalised eruption. There were large confluent patches all over the face, trunk, perineum, and limbs. There was also a large primary ulcer on the scrotum 3 inches in diameter, with very foul discharge. The patient was debilitated, and three doses of 1 gr. were given. There was some improvement, but it was very slow, so a dose of 2 gr. was given, and all the lesions were completely healed in six days, except the ulcer, which was reduced to less than 1 inch in diameter.

8. Some patches on the face with thick raised limpet crusts, and many small lesions on the scrotum, perineum, and buttocks, with moist discharging surfaces. Treatment, one dose of 1½ gr., followed by two doses of 2 gr. and a fourth of 1½ gr. All healed in 10 days.

9. Small patches on scrotum, penis, and perineum. Treatment, one dose of 1½ gr. and two of 2 gr. All healed in 10 days.

10. Small patches on scrotum, penis, perineum, and axillæ. Treatment, one dose of 1½ gr. and two of 2 gr. All lesions healed in 11 days.

In all cases the lesions were characteristic of the various stages of yaws, from minute vesico-pustules up to confluent, crusted lesions, rupioid patches with limpet-shell crusts, or late plantar-ulcers.

The diagnosis was confirmed in the earlier cases by examination of scrapings from the deeper parts of the lesions. Films were examined by dark ground illumination and spirochætes were found, sometimes in large numbers.

After the first dose the discharging ulcers showed signs of drying up and they skinned over rapidly. In 48 hours a distinct improvement was seen; the crusted lesions had shrunk somewhat and no longer contained fluid. The yellow colour disappeared and was replaced by a pearly grey; the underlying raw surface healed very quickly, and soon there was only a desquamating flake representing the site of the lesion. Accompanying the local changes there was improvement in the patient's general condition.

The number of cases treated is small, but they have been uniformly successful. The treatment can easily be carried out on a large scale, and it may be possible to cure large numbers of persons affected with this most unsightly, and hitherto long-enduring disease, without causing too great a drain on the funds allotted to medical work in these colonies, etc., where this condition is prevalent.

This preparation of antimony was tried successfully in a few cases of syphilis* by intramuscular injection, but the pain was so severe that the method was not continued. I venture to suggest that, as intravenous injection of the metal has proved feasible, a further trial may be warranted in this other spirochætal disease so closely allied to yaws.

The Liberation of Ions and the Oxygen Tension of Tissues during Activity. (Preliminary Communication.)

By H. E. Roaf, M.D., D.Sc.

(Communicated by Prof. C. S. Sherrington, F.R.S. Received January 10,—Read February 20, 1913.)

(From the Physiology Laboratory, St. Mary's Hospital Medical School.)

The hypothesis, that when cells become active ions are liberated, is frequently quoted in physiological writings, but measurements of the ionic changes are not given. The present research is an attempt to measure the ionic changes during tissue activity. A knowledge of these changes is desirable in order to compare the action of ions on colloids outside the cells with the changes that occur inside the cells.

On turning to the physiological literature one finds that little or nothing is given in the way of measurement of the ionic concentrations with which biology has to deal. Thus, neither the recent work on electrobiology by Bernstein,† nor that on the hydrogen ion in biological processes by Sorensen‡ contains any reference to the measurements of ions in tissues during rest or activity. Galeotti has, however, measured the hydrogen ion in heart muscle.§

Method.

The method used in the present series of experiments was to prepare a frog's sartorius muscle and arrange it for direct stimulation from an induction coil. A high resistance reflecting galvanometer was used and the muscle was tetanised. The muscle was tested with non-polarisable (Ringer solution, calomel) electrodes. Two of these were placed to touch the muscle directly opposite each other and the muscle was stimulated. If there was no definite deflection of the galvanometer, the cross-section of the muscle was considered to be iso-electric. Some other form of electrode was then substituted for one of the calomel electrodes and the muscle was stimulated. In order to avoid any influence of the stimulating current, the direction of the current in the primary coil was reversed and the stimulation was repeated.

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* 'Roy. Soc. Proc.,' B, vol. 80, p. 481.

† J. Bernstein, 'Electrobiologie' (Braunschweig, Vieweg und Sohn), 1912.

‡ S. P. L. Sorensen, 'Ergebnisse der Physiologie,' 1912, vol. 12, pp. 393–532.

§ G. Galeotti, 'Archivio di Fisiologia,' 1904, vol. 1, p. 512; 'Zeits. f. Allge. Physiol.,' 1906, vol. 6, p. 99.

Chlorine Ions.

The electrode used to replace one of the calomel electrodes was a silver wire coated with silver chloride. The arrangement was

$$\text{Ag} \mid \text{AgCl} \mid \text{muscle} \mid \text{Ringer solution} \mid \text{HgCl} \mid \text{Hg}.$$

This arrangement gave an electrical potential which was compensated in the usual way, and from the amount of compensation it was possible to calculate the concentration of chlorine ions in contact with the silver chloride–silver wire. After the compensation was accomplished the muscle was stimulated, and the direction of the galvanometer deflection was noted. The results showed that the silver electrode became more negative than it was with the resting muscle.

There are two contacts where the potential may be produced; the contact Ringer solution-HgCl-Hg not being affected by the contraction of the muscle.

The first is the contact Ag-AgCl-muscle, and an increase in chlorine ions would produce the increase in negativity when the muscle contracts.

The second contact is that between muscle and Ringer solution, and the potential would depend on the relative ionic mobilities of the positive and negative ions set free. For a binary electrolyte the formula would contain the ratio $(u - v)/(u + v)$, where $u =$ the rate of migration of the positive ion, and $v =$ that of the negative ion. A positive ion diffusing away from the muscle more rapidly than the negative ion would cause the mercury electrode to become positive, that is, the silver would become negative.

The only positive ion that need be considered is the hydrogen ion, as that is the only one that would give an appreciable value for $(u - v)/(u + v)$, and it will be shown in the next section that hydrogen ions are liberated when muscle contracts.

Against the possibility that when the muscle contracts the negative charge on the silver electrode is due to hydrogen ions diffusing into the Ringer solution, it may be pointed out that a saline electrode in contact with the active portion of a muscle becomes negative. Hence there may be a potential opposed to that due to the chlorine ions, and perhaps the action current may be added to the observed potential to give the true potential at the silver electrode. If, however, the usual electrical change is due to an increased permeability of a membrane to negative ions both silver and calomel electrodes will be equally affected, and hence the Ringer solution may become positive from diffusion of hydrogen ions in my experiments, whilst with the usual arrangement of electrodes the negative charge at the membrane overbalances that due to the diffusion of the hydrogen ion. The potential due to diffusion of the hydrogen ion should be reduced to a minimum by excess of indifferent electrolyte in the Ringer solution and muscle lymph.

Hydrogen Ions.

Galeotti (*loc. cit.*) used a hydrogen electrode to measure the hydrogen ion, but there is one objection to this electrode, namely, that if the electrode and the tissue in contact with it are saturated with hydrogen the behaviour of the tissue may be abnormal. To avoid this difficulty I have used a different form of electrode. Galeotti added the action potential to his observed potential, but until we know the true cause of the action current we cannot decide whether or no this addition is legitimate.

The oxygen electrode was not applicable and the reason for this will be found in the third section of this paper.

The electrode used to replace one of the calomel electrodes was a platinum wire covered with manganese dioxide.* The arrangement of the electrodes was

$$\text{Pt} \mid \text{MnO}_2 \mid \text{muscle} \mid \text{Ringer solution} \mid \text{HgCl} \mid \text{Hg}.$$

Using this arrangement and compensating as before, on stimulating the muscle the platinum electrode showed a very strong positive potential; so marked was the effect that a deflection was obtained by a single break shock. This cannot be due to negative ions diffusing into the Ringer solution, because in the ratio $(u-v)/(u+v)$ the hydroxyl ion is the only one that would give any appreciable negative value and the liberation of hydroxyl ions would be more effective in making the manganese dioxide electrode negative. Therefore, it is evident that hydrogen ions are liberated during muscular contraction.

Oxygen Tension.

Many text-books contain the statement that there is no oxygen tension in tissues. The author has pointed out that there must be some oxygen tension† and Verzár has shown, by an indirect method, the limiting values for submaxillary gland and muscle.‡

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* O. F. Tower, 'Zeitschr. f. physik. Chem.,' 1900, vol. 32, p. 566.

† H. E. Roaf, 'Brit. Med. Journ.,' September 28, 1912.

‡ F. Verzár, 'Journ. Physiol.,' 1912, vol. 45, p. 39.

The electrode used to replace one of the calomel electrodes was a piece of platinum wire covered with platinum black. The arrangement was

Pt | muscle | Ringer solution | HgCl | Hg.

The platinum electrode in oxygen or air gave results which when the muscle contracted showed sometimes a positive potential, sometimes after a slight positive potential a negative potential, and sometimes only a negative potential. The formula for the potential at a gas electrode contains the ratio $\sqrt[n]{P/p}$, where $P =$ the partial pressure of the gas and $p =$ the osmotic pressure of the corresponding ion. From this ratio it can be seen that a fall in oxygen tension would produce the same effect as a rise in hydroxyl* (fall in hydrogen) ion concentration. Therefore, there are two factors to consider, an increase in hydrogen ions, which tends to make the platinum positive, and a fall in oxygen tension, which tends to make it negative. The deflections of the galvanometer would, therefore, be explained as a rise in hydrogen ion concentration which is frequently overbalanced by a fall in oxygen tension.

The proof of this is very easy. When the access of air is prevented by a piece of rubber sheeting placed over the platinum wire as it lies against the muscle, the galvanometer may not show any deflection, but on stimulating the muscle the platinum becomes negative, thus indicating a fall in oxygen tension. This experiment has been frequently repeated and it is possible to convert one form of reaction into another. When the electrode is exposed to air the fall in oxygen tension is less, and hence, especially at the beginning of stimulation, the increase of hydrogen ions causes the platinum to become positive, but when the electrode is covered the fall in oxygen tension is greater, so that the rise of hydrogen ions is masked and the platinum *always* becomes negative. It is evident that this result may form the basis of a method for the direct measurement of oxygen tensions in tissues.

The results so far obtained are purely preliminary. Measurements of the time relations and the actual potential produced will show whether the liberation precedes, accompanies or succeeds the muscular contraction and will give some approximation to the relative increase in ionic concentrations.

Finally, it must be pointed out that the concentrations of ions measured are those in the lymph on the surface of the muscle. An increase may be due to an actual increase in ionic concentration inside the muscle or to an increase in permeability of a membrane previously impermeable to the ion measured.

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* Since the concentration of the doubly charged oxygen ion is proportional to the square of the hydroxyl ion concentration.

Reciprocal Innervation and Symmetrical Muscles.

By C. S. SHERRINGTON, F.R.S., Professor of Physiology, University of Liverpool.

(Received November 13, 1912,—Read January 23, 1913.)

(From the Physiology Laboratory, University of Liverpool.)

I. *Introduction.*

If we attempt to decipher the biological meaning of reciprocal innervation its various instances when marshalled together say plainly that one of the functional problems which it meets and solves is mechanical antagonism. Where two muscles have directly opposed effect on the same lever, "reciprocal innervation" is the general rule observed by the nervous system in dealing with them, and this holds whether the reciprocal innervation is peripheral as with the antagonists of the arthropod claw, or is central as with vertebrate skeletal muscles. Also where one and the same muscle is governed by two nerves influencing it oppositely, reciprocal innervation seems again the principle followed in the co-ordination of the two opponent centres, as has been shown by Bayliss* in his observations on vasomotor reflexes.

But the distribution and occurrence of reciprocal innervation extend beyond cases of mere mechanical antagonism. The reflex influence exerted by the limb-afferents on symmetrical muscle-pairs such as right knee-extensor and left is reciprocal.† Thus right peroneal nerve excites the motoneurones of left vastocrureus, and concomitantly inhibits those of the right. The reflex inhibition of the one is concurrent with, increases with increase, and decreases with decrease of, the excitatory effect on the other. Here the muscles are not in any ordinary sense antagonistic; not only do they not operate on the same lever, but they are not even members of the same limb, nor do they belong even to the same half of the body. They are, however, actuated conversely in the most usual modes of progression—the walking and the running step—though not always in galloping.

Similarly with other symmetrically paired limb muscles,‡ the limb afferents when tested for their reflex effect on such twin muscles commonly exert an opposite and reciprocal effect on the members of the pair. Here the bifurcation of the afferent path which leads to the reciprocal effect sits, so to say, astride of the median longitudinal plane of the body, and a somewhat analogous case is that of the reciprocal innervation exerted by *cortex cerebri* on certain symmetrical muscle pairs in, for instance, the musculature of the eyeballs.* Stimulation of a point in the right cortex, while causing left external rectus to contract, causes right external rectus to relax, and conversely inhibits left internal rectus while exciting contraction of right internal rectus.

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* 'Roy. Soc. Proc.,' 1908, B, vol. 80, p. 339.
† 'Journ. Physiol.,' 1898, vol. 22, p. 398.
‡ 'Roy. Soc. Proc.,' 1905, B, vol. 76, p. 286.

But in the case of such symmetrical muscle-pairs, though some circumstances and some afferents deal with the two members of the pair by "reciprocal innervation," it is equally clear that some deal with them by "identical innervation." Thus with the two vastocrurei, right and left, though most of the limb-afferents influence the two reciprocally, the genito-crural nerve influences them identically,† namely, excites concurrent contraction of both muscles, and it is clear that in their natural use both the muscles are sometimes thrown into contraction together and thrown out of contraction together, as happens in the gallop and in standing and sitting. Similarly with the lateral recti of the eyeballs, stimulation of certain brain points and certain voluntary acts which cause ocular convergence exert an identical influence on the two internal recti exciting both together, and similarly an identical influence on the two external recti inhibiting both. The relation of reciprocal innervation to the symmetrical muscle-pairs differs, therefore, from its relation to antagonistic muscles, in so far that in the former case it is only one of the ordinary modes of innervation obtaining for the muscle-pair, whereas in the latter case there is little evidence at present that reciprocal innervation of the muscle-pair is at all commonly under normal circumstances replaced by identical innervation.

Such symmetrical muscle-pairs present, therefore, the problem that sometimes they are co-ordinated by reciprocal innervation and sometimes by identical innervation. In the present observations it has been sought to see whether by experimental means in the purely reflex preparation they can be made sometimes to contract together or to relax together and at other times to behave reciprocally, the one member of the pair contracting concomitantly as the other relaxes.

II. *Change from Reciprocal Innervation to Identical in Symmetrical Extensors.*

When an afferent nerve of one hind limb is stimulated in the double (right and left) vastocrureus preparation (decerebrate cat), reciprocal innervation is seen to obtain in the reflex effect on the two muscles. The muscle contralateral to the nerve exhibits excitatory contraction; the ipsilateral muscle inhibitory relaxation (fig. 1). This result holds good over a wide range of intensities of stimulation of the afferent nerve. The contralateral excitation and the ipsilateral inhibitory relaxation increase *pari passu* as the intensity of the stimulation is increased.

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* 'Roy. Soc. Proc.,' 1893, vol. 52, p. 333.

† 'Journ. Physiol.,' 1910, vol. 40, p. 53.

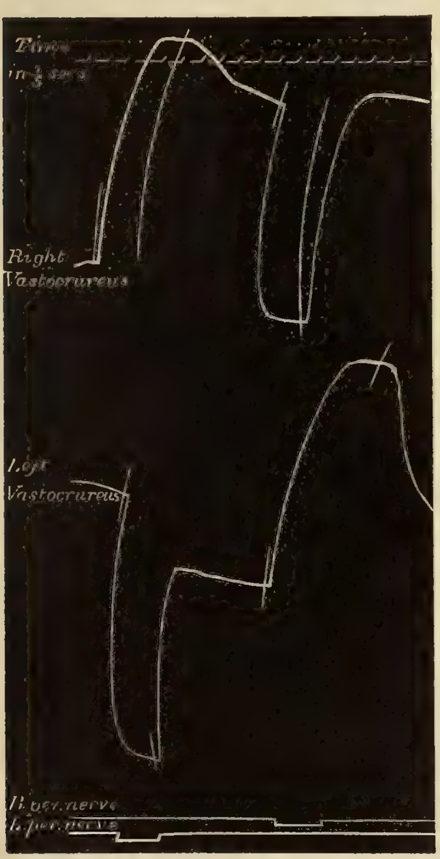

Fig. 1.

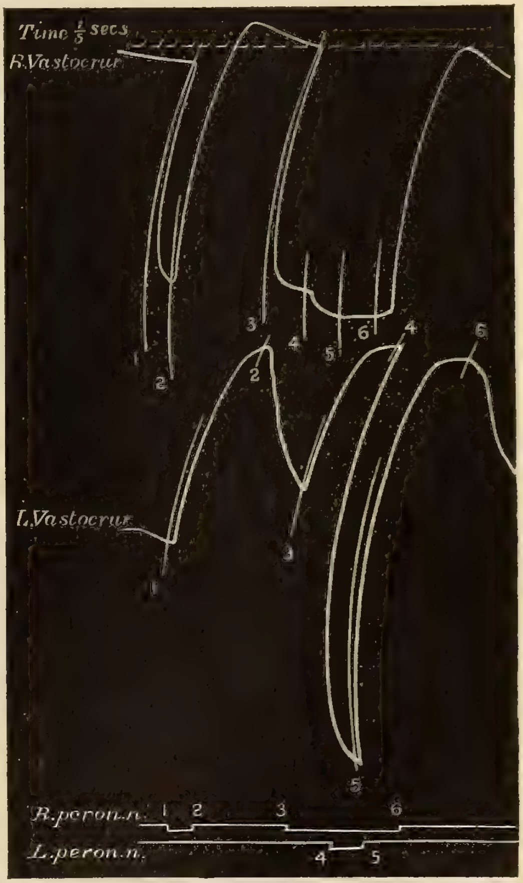

Fig. 2.

Fig. 1.—Reciprocal innervation of the extensor muscles, *vastocrurei*, of right and left knee, first from left peroneal nerve, then from right peroneal. Decerebrate cat. The inhibitory relaxations are in each case followed by post-inhibitory rebound.

Fig. 2.—Extensor muscles, *vastocrurei*, of right and left knee. The right peroneal nerve is first stimulated; then the same nerve again, and during its stimulation left peroneal is stimulated with same intensity approximately as right, and the left stimulus withdrawn, and finally the right. During the double stimulus both muscles exhibit inhibitory relaxation. Decerebrate cat.

But it is also possible experimentally to obtain simultaneous inhibitory relaxation of both muscles followed by simultaneous contraction of them. If the preparation be carefully made, the condition of the twin isolated muscles remaining sensibly similar, and if similar right and left afferent nerves, *e.g.* right and left peroneal, be fitted with electrodes from similar induction coils similarly supplied and interrupted for faradisation and the intensity of the stimulation of the two nerves be kept as far as practicable equal, observations can be obtained as follows:—Suppose, as in fig. 2, right peroneal stimulated; right vastocrureus relaxes and left contracts; if then, while right nerve continues to be stimulated, left peroneal be stimulated in addition, right vastocrureus still remains relaxed and indeed may relax further, but left vastocrureus relaxes also. On then withdrawing the stimulation of left nerve left muscle contracts and right remains relaxed still, and finally on withdrawal of stimulation of right nerve right muscle contracts by rebound.

This shows that each of the afferent nerves employed taken by itself unfolds in response to stimulation an inhibitory effect on the ipsilateral muscle stronger than is its excitatory effect on the twin contralateral muscle. Stimulation of the right and left nerves concurrently if the stimulations be fairly equal in intensity causes therefore concurrent relaxation of both muscles. Thus when the stimulations of the two nerves are repeated synchronously, as in fig. 3, both muscles relax together at each repetition of the stimulation. Further at each discontinuance of the double stimulation both muscles exhibit synchronous rebound contraction. So that in response to the synchronously repeated and synchronously remitted stimulations both muscles relax and contract synchronously. If, however, the intensities of the two stimuli be markedly unequal the muscular reactions, right and left, though synchronous are, of course, reciprocal, not identical.

The identical form of reaction holds true over a wide range of intensities of stimulation, so long as the two stimulations, right and left, are kept of approximately equal intensity. The preponderance of ipsilateral inhibition over contralateral excitation obtains therefore both with moderate stimuli and with strong, the ratio between the intensities of the reflex inhibition and reflex excitation remaining apparently about the same for a wide range of stimulus intensities. The difference between the effect of synchronous double stimulation of strong intensity and of weak is in the main merely that with weaker stimuli the synchronous relaxations of the two muscles are weaker and are followed by less powerful rebounds than are strong, though the rebounds are still synchronous.

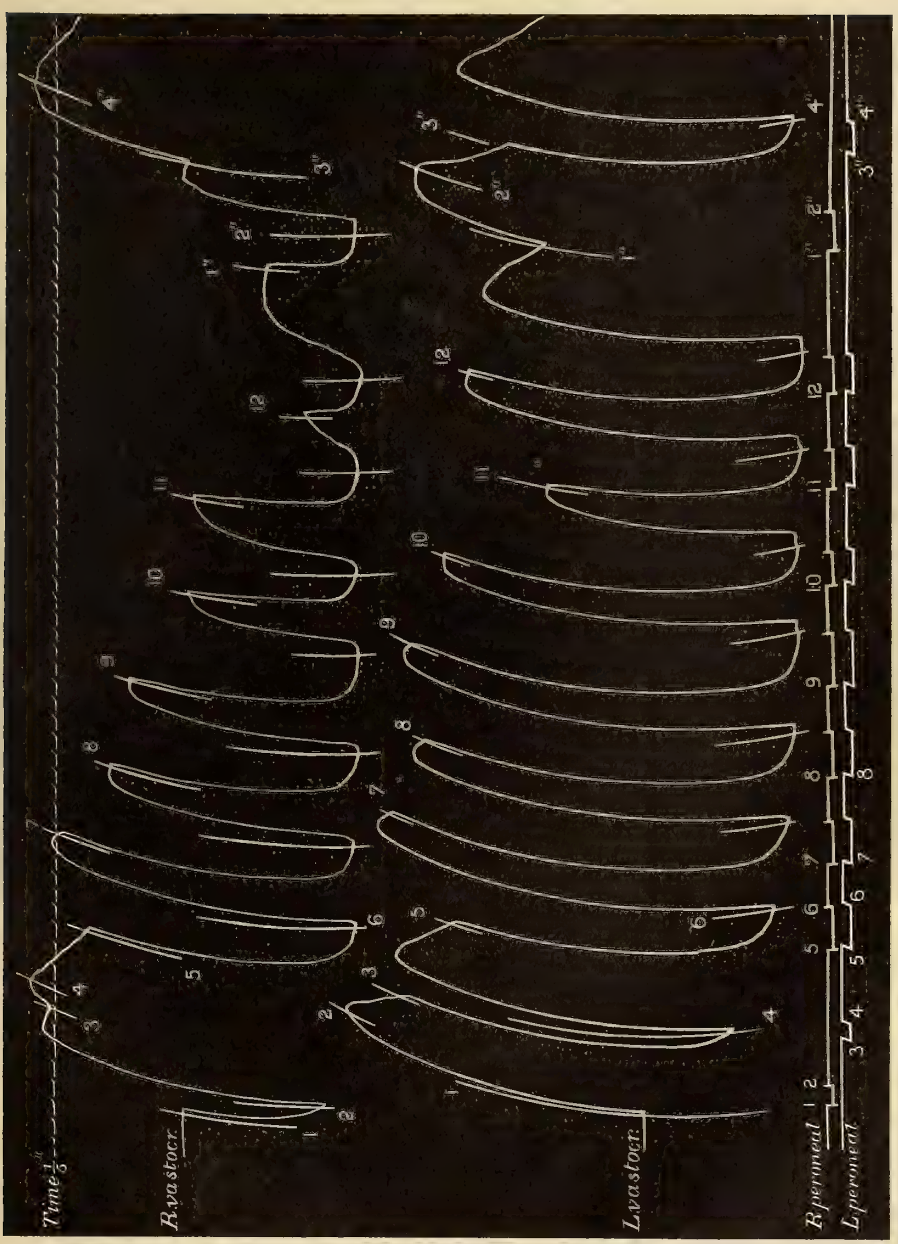

Fig. 3.—Extensor muscles, *vastocrurei*, of right and left knee. Stimulation of right peroneal, then of left, then seven stimulations of both nerves together, then of right alone, and finally of left alone. The intensities of stimulations right and left approximately equal. Decerebrate cat.

III. *Change from Reciprocal Innervation to Identical in Symmetrical Flexors.*

If instead of extensor muscles we take a pair of symmetrical flexors, upon them again reciprocal innervation is found to be the reflex result of stimulation of an afferent nerve of either limb. Thus with the two tensor fasciæ femoris muscles, hip-flexors, the effect of stimulation of the central end of the peroneal nerve is contraction of the ipsilateral muscle and inhibitory relaxation of the contralateral (fig. 4). So also with psoas, and sartorius (fig. 5), and semitendinosus, the latter a flexor of knee. Yet with these flexors, although thus reciprocally innervated, it has been shown that, under certain circumstances, they exhibit identical reflex innervation, for instance when nociceptive stimulation is applied concurrently to both feet.* And when the two peroneal nerves right and left are concurrently stimulated with faradisation of approximately equal intensity, the two flexor muscles right and left both contract together (fig. 6) and exhibit fully identical reflex innervation. Here, however, the identical innervation presents itself in the form of concurrent contraction not of concurrent relaxation as was the case with the extensors. With the flexors, therefore, the excitatory effect of each

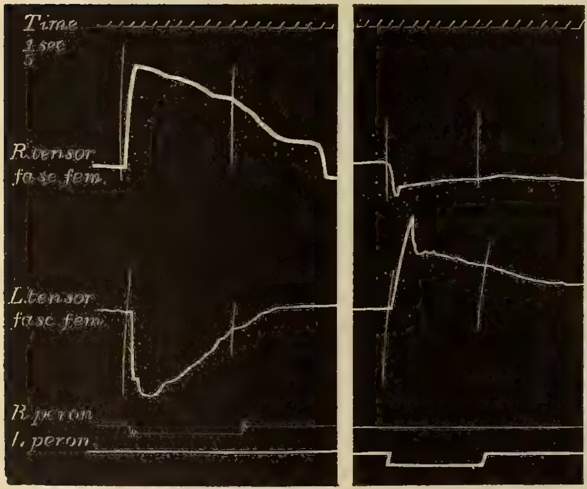

FIG. 4.

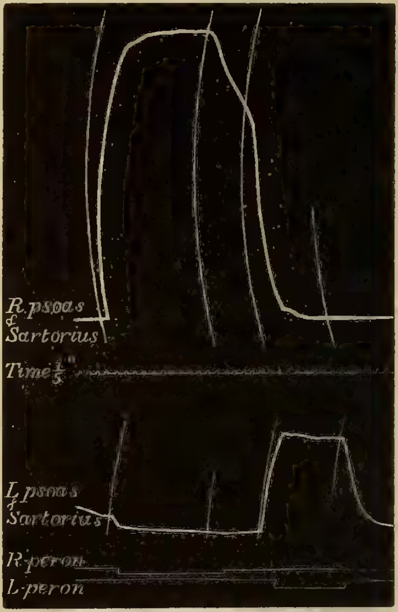

FIG. 5.

FIG. 4.—Flexor muscles, *tensor fasciæ femoris*, of right and left hips. Stimulation of right peroneal nerve, and then of left peroneal nerve. Decerebrate cat.

FIG. 5.—Flexor muscles, *psoas* and *sartorius*, of right and left hips. Stimulation of right peroneal causes contraction of the right muscles and inhibitory relaxation of the left. Stimulation of the left peroneal causes inhibitory relaxation of the right muscles and contraction of the left. Decerebrate cat.

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* Sherrington, in 'Schäfer's Handbook of Physiol.,' 1900, vol. 2, p. 840; 'Integrative Action of Nervous System,' p. 225, 1906.

afferent is stronger than the inhibitory effect, and the excitatory effect is ipsilateral. So that with both flexors and extensors the ipsilateral effect is the stronger, but in the case of the flexors it is excitatory while with the extensors it is inhibitory.

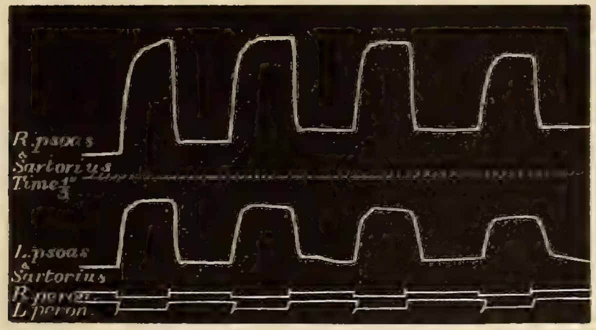

Fig. 6.—Flexor muscles, *psoas* and *sartorius*, of right and left hips. Stimulation of the right and left peroneal nerves together causes contraction of both the right and left muscles synchronously. The stimulation intensities right and left were approximately equal. Decerebrate cat.

IV. *Algebraic Summation as a Factor in Producing the Change.*

In the case of these symmetrical muscles therefore the change from reciprocal reflex effect to identical is clearly explicable by algebraic summation of excitation and inhibition.* The result may be stated numerically. Right peroneal nerve under a stimulus whose intensity may be figured as 10, causes an excitation of the motoneurones of right flexor muscle of an intensity expressible as 10, and a weaker inhibition of the motoneurones of left flexor whose intensity may be called 6. It at the same time causes also an inhibition of the motoneurones of the right extensor of intensity 10, and a weaker excitation of the motoneurones of left extensor of intensity 6. Similarly, the reflex effect of the left peroneal nerve under a stimulus of like intensity is on the left flexor's motoneurones an excitation of value 10, and on right extensor's motoneurones of value 6, while left extensor's motoneurones it inhibits with value 10 and right flexor's motoneurones with value 6. Denoting (fig. 7) excitation by the prefix + and inhibition by the prefix −, the resultant reflex effect on the motoneurones of the muscles severally is, when both nerves are stimulated concurrently, + 4 for the flexor in each limb, and − 4 for the extensor in each limb. So that, if we suppose all the muscles to be previously in a condition of medium tonus the two symmetrical flexors then contract and the two symmetrical extensors exhibit inhibitory relaxation. It is to be noted that if the initial condition of the muscles be full repose, *e.g.* −10 in the above notation, then in the above instance the symmetrical extensors (as well as the flexors) enter upon a certain degree of contraction, namely −4, full relaxation being −10. In any case the double stimulus gives an identical reflex effect on the symmetrical muscles, though the reflex effect still remains reciprocal as regards the pairs of antagonistic muscles. Under the double stimulus the reflex effect becomes symmetrical and identical in the two limbs, although under either of the two components singly the reflex effects in the two limbs are diametrically opposed.

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* 'Roy. Soc. Proc.,' 1908, B, vol. 80, p. 565.

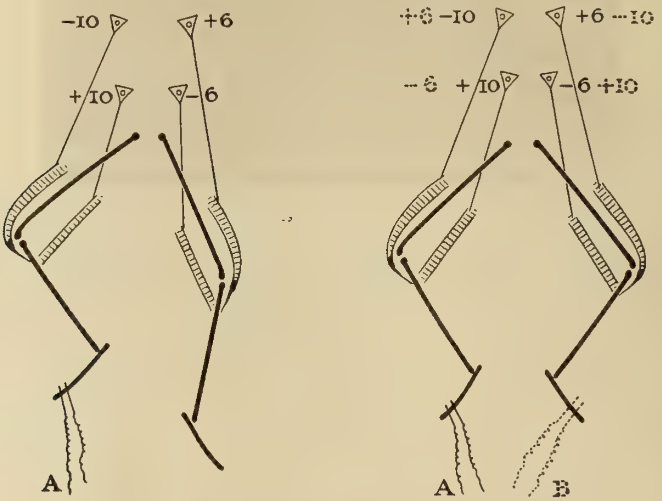

Fig. 7.—Explanation in text.

These results hold over a wide range of intensity of stimuli so long as the intensities of the stimuli right and left are approximately equal. This indicates that the intensity ratio between the ipsilateral and contralateral effects, both of inhibition and excitation, remains but little changed over a wide range of different intensities of stimulation. The absolute values rise and fall with increase and decrease of stimulation, but the relative values remain about the same, subject to two alterations which will be mentioned later.

The above observations therefore come under the rubric of algebraic summation in double reciprocal innervation. They show that double reciprocal innervation can change reciprocal innervation of *symmetrical* muscles into identical innervation of them. Double reciprocal innervation* applied to *antagonistic* muscles does not result in identical innervation of them, although it can bring about, it is true, the exhibition of some degree of contraction by both the antagonists at the same time.

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* 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 249.

The difference in intensity of reflex influence exerted on the ipsilateral and contralateral limbs respectively may have its biological meaning in the opportunity thus given for the limbs to exhibit either symmetrical reflex movements, or movements of opposed direction right and left, according as there is equality or inequality between their right and left stimuli.

V. *Symmetrical Rebounds.*

It will be noted that in the above attempt to change by experimental means the reciprocal innervation of the symmetrical muscle-pair consisting of right and left knee-extensor into identical innervation, success is reached as regards "immediate"* reflex effect only in so far as identical inhibitory relaxation of the two. Simultaneous contraction of the two in response to concurrent stimulation occurs occasionally with weak stimuli, especially where one of the nerves tends to give ipsilateral contraction. The identical contraction of the two which ensues, however, after their concomitant relaxation is, so to say, not an immediate but a "terminal"† reflex result, for it is due to post-inhibitory rebound.

Rebound contraction in one muscle of an antagonistic or of a symmetrical pair is so commonly associated with concomitant relaxation of the fellow muscle (fig. 8) of the pair that large identical rebound contractions

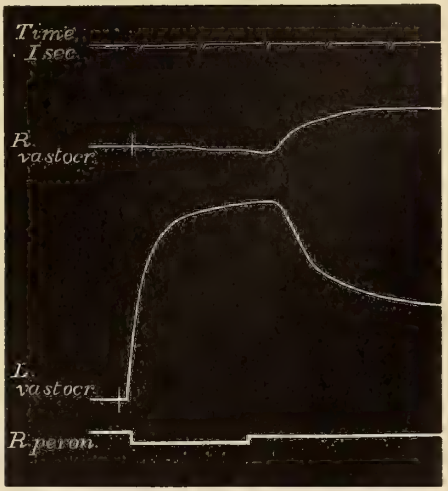

Fig. 8.—Extensor muscles, *vastocrurei*, of right and left knees. Stimulation of right peroneal nerve. The post-inhibitory rebound of the ipsilateral extensor is synchronous and reciprocal with the post-excitatory relaxation of the contralateral extensor. Decerebrate cat.

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* *Cf.* T. Graham Brown, 'Quart. Journ. Exp. Physiol.,' 1912, vol. 5, p. 237.

† *Cf.* T. Graham Brown, 'Quart. Journ. Exp. Physiol.,' 1911, vol. 4, p. 331.

occurring concomitantly in the symmetrical muscles is not without interest. T. Graham Brown* has recently pointed out that in antagonistic muscles in many cases the terminal relaxation following an excitatory reflex may be regarded as of the nature of an inhibitory rebound, the converse of rebound contraction. It would seem, therefore, that the terminal rebound following a reflex of reciprocal effect on a muscle-pair is often itself of reciprocal character in the two muscles. In the symmetrical muscles dealt with in this paper the terminal effects when the reflex itself has been of reciprocal influence on the two muscles are quite usually of reciprocal character in the two muscles (see fig. 8). When, however, the character of the reflex itself has been changed, by the procedure described, from reciprocal into identical the terminal rebound also is changed from reciprocal into identical (fig. 3).

VI. *Factors Outside Algebraic Summation Involved in the Change.*

The above seems to me what the experiments clearly indicate as the main principle involved in the change from reciprocal innervation of symmetrical muscles to identical innervation of them when the stimuli are appropriately duplicated. This principle rests on the inequality of the excitation-potency and inhibition-potency respectively inherent in the components of the summed duplicate reflex. But the experiments have shown certain further features outside this principle. In the observations on the vastocrureus muscles, when the reflex inhibition due to the ipsilateral nerve is in progress, and stimulation of the contralateral nerve is then added, the effect of the latter is very occasionally not a mitigation but a distinct increase of the inhibitory relaxation (fig. 2, abscissæ 4, 5). A similar result is sometimes met with in the flexor, tensor fasciæ femoris, though there conversely in regard to the nerves used. A phenomenon comparable with these, although in the opposite direction, was reported previously by Miss Sowton and myself working with the knee-flexor, semitendinosus.† We noted that if the stimulation of contralateral nerve is relatively weak in comparison with that of the ipsilateral nerve, the former, if added when the latter is in progress, may, instead of lessening the reflex contraction due to the latter, actually increase it. In the observations of this paper a strong reflex inhibition of the extensor centre already in progress seems to convert a weak excitatory influence into an inhibitory one. In the previous observations a strong reflex excitation of the flexor centre seemed to convert a weak inhibitory influence into an excitatory one. And it has been the case in some of the present observations on the hip-flexors that the addition of a strong contralateral stimulus to an already existent ipsilateral has increased instead of lessened the contraction (fig. 9), and this has been so not only during the application of the contralateral stimulus but still further on its withdrawal. A rebound contraction of the flexor muscle has then occurred, adding itself to the contraction due to ipsilateral nerve, and causing a combined contraction of large amplitude.

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* *Ibid.*

† 'Roy. Soc. Proc.,' 1911, B, vol. 84, p. 204.

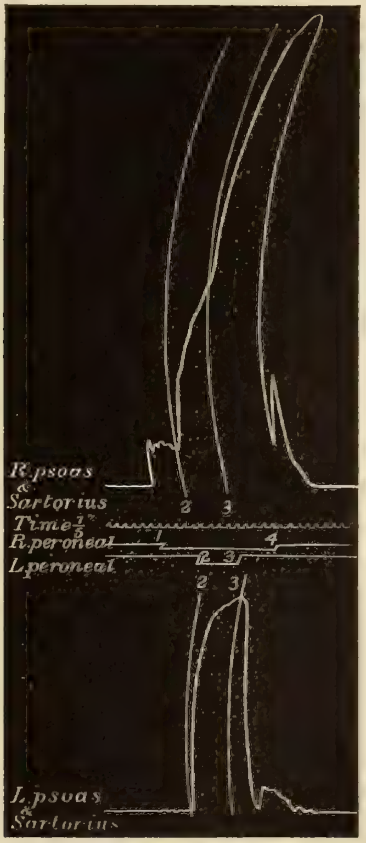

FIG. 9.—Psoas and sartorius muscles, flexors, of right and left limbs. Stimulation of right peroneal with a weak stimulus, coil at 24 cm. from primary; during this stimulation the left peroneal is stimulated for about one second with a strong stimulus, coil at 14 cm. from primary. The contraction of right flexors is increased during stimulation of left nerve, and on cessation of this stimulation a still further rebound augmentation of the right muscle's contraction occurs. Decerebrate cat.

It is obvious that such reactions though outside the main principle above stated work in the direction of assisting a double reciprocal innervation to change the reflex effect on the symmetrical muscles from reciprocal to identical character. The contraction of both flexors will be increased and so also the inhibitory relaxation of both extensors.

It seems, therefore, that in the combination of two reciprocal reflexes of opposite effect on the symmetrical muscles of the limbs there is, besides simple algebraic summation of the respective excitation and inhibition of the components, a further factor sometimes present. An actual reversal of the weaker element of one of the components appears to take place. Thus, the excitatory effect on the contralateral extensor tends occasionally to be reversed to inhibitory effect, and the inhibitory effect on contralateral flexor tends to be reversed to excitatory effect.

There remains the question whether identical concurrent contraction of the symmetrical extensor pair can be obtained as a direct reflex. With concurrent stimulation of both nerves, right and left, it can occasionally, when the stimulation is weak, especially if one or both nerves be then giving ipsilateral extensor contraction. Experiment shows that it can also be obtained by a procedure quite other than that followed for producing the identical innervation which has symmetrical relaxation for its result, namely by weak stimulation of the afferent nerve of one side alone, right or left. The range of intensity of stimulus over which reciprocal innervation of the muscle pair results from excitation of a limb afferent is wide and ranges from weak intensities without break right up to the very strongest. But with stimuli of little above threshold value the result changes in the decerebrate preparation. With these stimuli although the response in the contralateral muscle remains reflex contraction, in the ipsilateral muscle the response becomes reflex contraction instead of inhibitory relaxation. This alteration is that noted previously by Miss Sowton and myself.* That it is accompanied by concomitant contraction of the symmetrical extensor of the fellow knee shows that with change merely of intensity of the stimulus the reflex innervation of the fellow muscles alters from reciprocal to identical. The reflex contraction so obtained is weak but quite indubitable and clearly concomitant (fig. 10).

Over a range of stimulus-intensities running from threshold value up to weak or moderate, the exact limit upward varying with the condition of the preparation, the reflex result in the decerebrate animal passes from identical innervation (fig. 10, A) to an admixture of identical and reciprocal innervation (fig. 11), until with stronger stimuli reciprocal innervation is fully established (fig. 10, B). In the admixed form of result the reflex opens with bilateral contraction, *i.e.* contraction of both right and left extensor muscles, and then passes over into ipsilateral inhibitory relaxation, with contralateral pure contraction (fig. 11). This transition is speedier the less weak the stimulation. It is brought about by a change occurring, as noted by Miss Sowton and myself,* in the reaction of the ipsilateral muscle during the progress of the stimulation.

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* 'Roy. Soc. Proc.,' 1911, B, vol. 83, p. 435.

The statement was made above that the relative values of ipsilateral inhibition and contralateral excitation in the extensors remain about the same over a wide range of intensities of reflex stimulation, although the

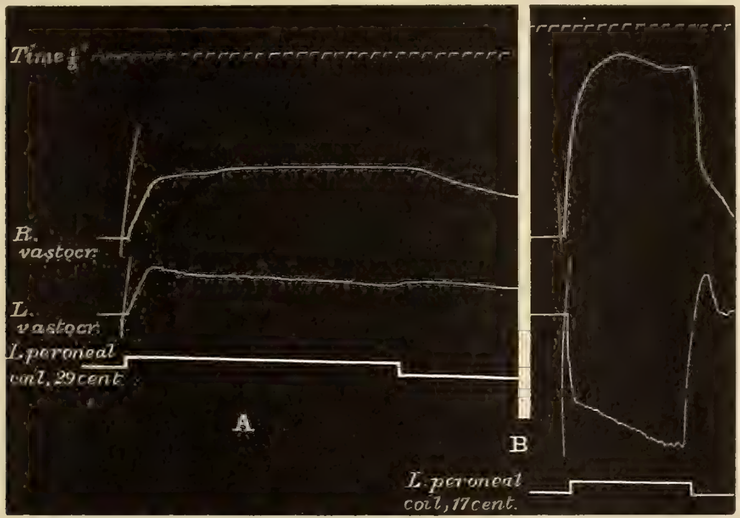

Fig. 10.—Extensor muscles, *vastocrurei*, of right and left knees. In the left-hand record the stimulation of left peroneal is weak and little above threshold value ; in the right-hand record the stimulation of the same nerve is repeated, but with increased though still moderate intensity. Decerebrate cat.

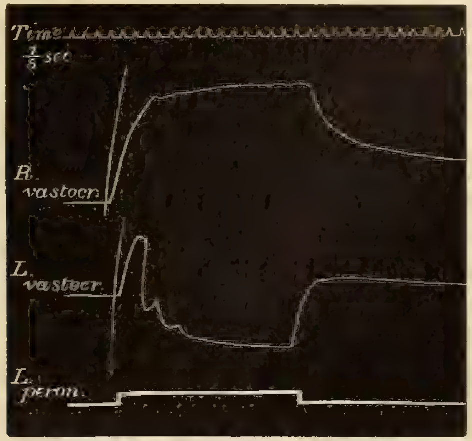

Fig. 11.—Extensor muscles, *vastocrurei*, of right and left knees. Stimulation of left peroneal with moderately weak faradisation. The reflex effect opens by being "identical" in the two muscles, but later changes to "reciprocal" as the stimulation continues. Decerebrate cat.

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* *Ibid.*

absolute values rise and fall *pari passu* with the stimulus intensity. What has just been said has to be remembered in relation to that statement. With decrease of stimulus intensity, as just said, a value of stimulus is ultimately reached, at which in the decerebrate preparation contralateral effect still remains excitatory, but ipsilateral becomes excitatory instead of inhibitory. The biological meaning of this may be that with these weak stimuli the reflex produced is that of standing, *i.e.* a local reflex contributory to the great compound reflex of standing, whereas, with stronger stimuli, the reflex produced is the nociceptive flexion reflex, or the flexion phase of a locomotor step-reflex.

With the flexor muscles of the hip, psoas, tensor fasciæ femoris, and sartorius, strong stimulation of one peroneal nerve sometimes excites contraction in these muscles in the contralateral, as well as in the ipsilateral limb. The contraction of the contralateral muscles is less strong than that of the ipsilateral. It tends to be followed on withdrawal of the stimulus by marked rebound contraction. The contralateral contraction of the hip-flexors recalls the contralateral contraction of the ankle flexor, tibialis anticus, noted by T. Graham Brown,* as sometimes occurring in both decerebrate and spinal preparations.

VII. *Summary of Conclusions.*

1. The occurrence and distribution of reciprocal innervation extends to cases of muscular co-ordination beyond those involving simple mechanical antagonism. Thus it is exemplified also in reflexes actuating symmetrical muscles, for instance, muscles symmetrically placed in the right and left limbs.

2. These muscles present the problem that, in reflexes, though often worked reciprocally, they are also often worked identically.

3. Experiments cited show certain ways in which the stimulations can be experimentally arranged to give either reciprocal or identical innervation of symmetrical muscles of right and left limb.

4. It is shown that algebraic summation of excitation and inhibition can explain this result.

5. It is further shown that there is evidence that other factors besides simple algebraic summation of the individual component reflexes have a share in changing the reciprocal innervation into an identical. A reversal of the weaker element of one of the components appears to occur. Thus, the excitatory effect on the contralateral extensor tends occasionally to be reversed to inhibitory effect, and the inhibitory effect on the contralateral flexor tends to be reversed to excitatory effect.

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* 'Journ. Physiol.,' 1912, vol. 44, p. 125.

Nervous Rhythm arising from Rivalry of Antagonistic Reflexes: Reflex Stepping as Outcome of Double Reciprocal Innervation.

By C. S. Sherrington, F.R.S.

(Received February 3,—Read February 20, 1913.)

(From the Physiological Laboratory, University of Liverpool.)

I.

The observations with which the present communication deals were met with in experiments continuing those on reciprocal innervation of symmetrical muscles. In my previous paper on that subject* it had been reported that in regard to symmetrical extensors of the knee the ratio borne by intensity of the ipsilateral inhibition to the contralateral excitation is such that with equal stimuli to right and left symmetrical afferent nerves there is inhibitory suppression of contraction in both the muscles. In other words, under double reciprocal innervation the ipsilateral inhibition by each nerve completely overcomes the contralateral excitation of the other. It was shown that this mutual suppression holds over a wide range of the scale of intensities of stimulation. It was also shown that with quite weak stimuli a simultaneous stimulation of both nerves, stimuli being equal in intensity, often results in concurrent contraction of both muscles. Indeed, with quite weak stimuli, the effect of stimulation of each afferent nerve by itself is, in the decerebrate preparation, usually contraction of the ipsilateral as well as of the contralateral muscle.

This being so, it is evident that at some point in the scale of intensities of stimulation there should be a place below which contralateral excitation is stronger than ipsilateral inhibition, whereas above it ipsilateral inhibition is stronger than contralateral excitation.

My further experiments were directed to finding where on the intensities-scale this point actually lies. In prosecuting this search there began to be met instances of rhythmic contraction of exceedingly pronounced character.

Method employed.

The mode of preparation when the knee extensor was observed was as follows:—The animal (cat) was decerebrated under deep chloroform and ether narcosis. In both limbs all muscles were then detached from the great and lesser trochanters and intertrochanteric line. The insertions of right and left iliopsoas, psoas parvus, and tensor fasciæ femoris were then carefully resected up, and the origin of rectus femoris right and left. In both limbs the following nerves were severed: popliteal, small sciatic, hamstring, external cutaneous, obturator, internal saphenous and branch to the sartorius and pectineus. The peroneal of each limb was ligated tightly at its entrance into tibialis anticus just below knee. This procedure leaves the vastocrureus muscle of each limb the muscle operative in or on the limb. A steel drill in the condylar end of each femur is clamped to a heavy upright in such a position that, the preparation being supine, the two femora are nearly vertical and are parallel, and both hips are flexed to about a right angle. The root of the tail is fixed to the table by a steel pin. The leg is removed one-third down the tibia, and into the free end of that bone a fine steel pin is set; to this the thread attached to the myograph lever is fastened. The angle made by the tibia with the thigh varies with the degree of contraction of vastocrureus, and, the preparation being decerebrate, that muscle exhibits tonus, *i.e.* maintains the standing posture.

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* *Supra*, p. 219.

The resistance to the contraction of each vastocrureus was provided by the weight of the remaining portion of the limb below the knee and the adjustable tension of a light coiled wire spring attached to the lever of the myograph near its axis. The resistance was made as nearly similar as possible for both the right and the left muscle. The peroneal nerve on each side was stimulated with platinum electrodes, 4 mm. apart and placed with the nerve-trunk obliquely between them. The pair of electrodes was inserted sidewise through a glass tubulure in the wall of a glass tube, in which lay the ligated nerve itself. The muscles and skin around and over the electrode and tube were brought together and stitched. For flexor observations the psoas and tensor fasciæ femoris were employed and isolated, the animal being prone.

For stimulation a pair of similar Leeds induction coils were used, one for the right nerve, the other for the left. In the secondary circuit of each a resistance box of 100,000 ohms was introduced. A double switch connected to the circuits for both nerves allowed the coils to be interchanged for the two nerves at will, furnishing some opportunity of testing the approximate equality of the two circuits and of the relative excitability of the two nerves.

The right and left vastocrurei, thus completely isolated in each limb, but retaining blood and nerve supply and natural attachments quite intact, form a symmetrical pair of extensor muscles. Under the reflex action of, for instance, an afferent nerve of either limb, reciprocal innervation obtains for them as it does for an antagonistic muscle-pair. As shown, the afferent of each limb relaxes the ipsilateral vastocrureus by reflex inhibition, and causes the contralateral to contract by reflex excitation. The motor centre of the contralateral extensor reacts therefore to the afferent in the same direction as does that of the flexor of an ipsilateral antagonistic pair.

For the observations on double reciprocal innervation required I employed symmetrical afferent nerves, usually the peroneals, sometimes the popliteals. With simultaneous stimulation of both right and left nerves algebraic summation of the excitatory and inhibitory effects is of course the result.* Over a certain range of combinations this summation is readily observable because it results in contractions not wholly suppressed but of various grades of submaximal intensity. Somewhere within this restricted range of combinations of the opposed stimuli should lie the neutral point sought

* *Vide supra*, p. 226.

for in my experiments. When pursuing adjustments of the stimuli in order to arrive at determination of this it was found that rhythmic alternating contraction and relaxation, reciprocal in direction in the two muscles, appeared directly both stimuli were employed concurrently, though quite absent under either stimulus alone.

During these rhythmic reactions a glance at the preparation was enough to show that, although only the one thigh muscle remained in each limb, the animal was stepping with both limbs, usually at a quick walking pace, and whether the muscle remaining were an extensor or a flexor. It began to step as soon as ever the two antagonistic stimuli were concurrently combined, and it ceased to step directly their union was dissolved. Fig. 1 exemplifies this. There it is seen that it did not matter whether the combination were made by adding the inhibitory stimulus to the excitatory already in action, or the excitatory to the previously-acting inhibitory; in either case the stepping did not occur under either stimulus acting alone, yet ensued directly the two opposed stimuli were in action together. Conversely, on withdrawing either of the stimuli and leaving the other one in operation the stepping immediately ceased, each limb then passing into either steady contraction or steady relaxation, according as the remaining stimulus was contralateral to it or ipsilateral.

II.

Nervous inhibition seems in many ways the exact opposite and counterpart of nervous excitation. And since the process of nervous excitation is certainly rhythmic, its natural rhythm in mammalian motoneurones being probably about 50 impulses per second (Piper),[*] it may be argued that the process of nervous inhibition is probably similarly rhythmic. Yet in comparison with the slow periods of many rhythmic muscular acts, for instance those of breathing and stepping, a rhythmic frequency of 50 per second can be considered as tantamount to continuous and steady operation. It is a question how there are developed from such minutely oscillatory nervous discharge those coarser rhythmic actions with periods recurring once in 3 secs. as in breathing, or once a second as in the step, or four times a second as in the scratch reflex. The suggestion has often been made that such rhythmic nervous actions are the result of the concurrent action of two opposed nervous forces, the outcome of a constant opposition or resistance acting against a constantly discharging nervous activity. "How can the constant motion of the nervous fluid be changed to a periodic motion? When a conductor of electricity is held at some distance from the electric

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[*] 'Elektrophysiologie menschlicher Muskeln,' Berlin, 1912.

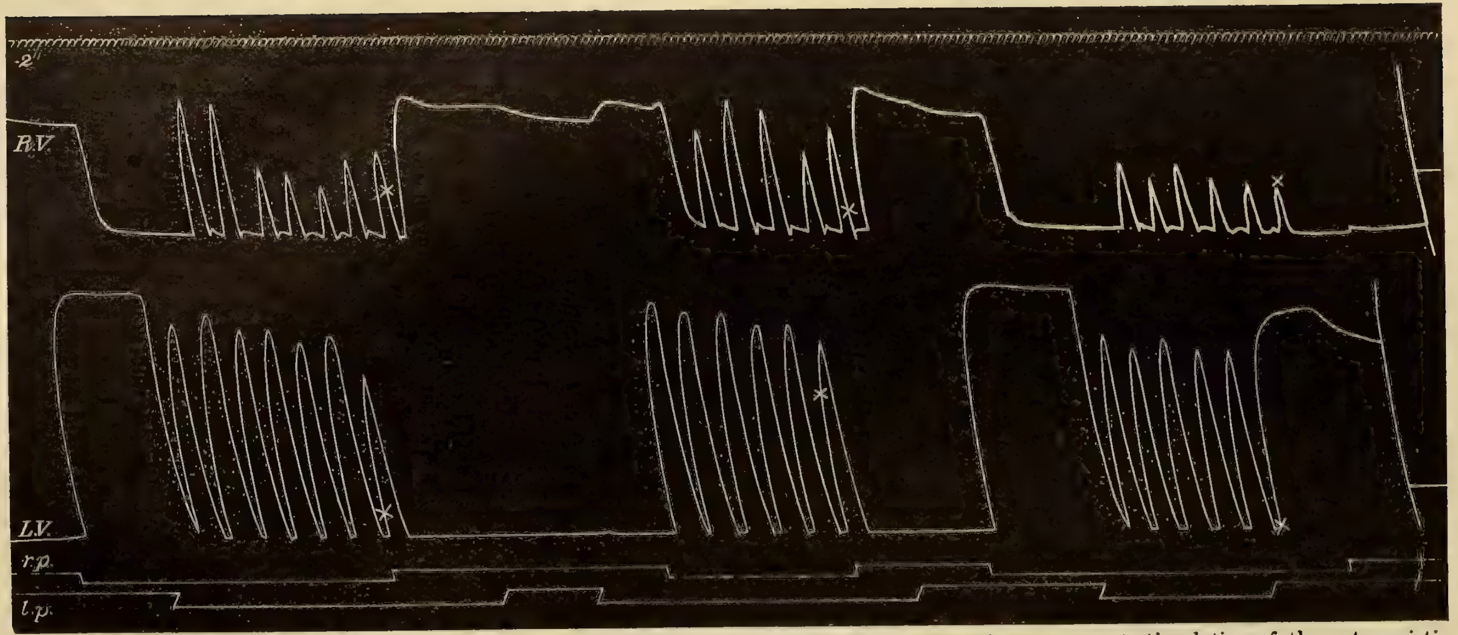

FIG. 1.—Reflex reciprocal stepping of isolated extensor muscle of knees, right and left, evoked by concurrent stimulation of the antagonistic nerve-pair, right peroneal (*r.p.*) and left peroneal (*l.p.*) used as afferents. Decerebrate cat. R.V., right vastocrureus muscle; L.V., left vastocrureus muscle. Time in fifths of seconds above. The small crosses mark the moments at which the combination of inhibitory and excitatory stimulation is broken by withdrawal of one or the other. Ascent of myograph line denotes contraction, descent relaxation. The preparation is a tonic one, so that descent of myogram line means active inhibitory relaxation. Further explanation given in text, p. 241.

machine, kept in a state of constant excitement, the electricity is given off periodically. The dry air between the machine and the conductor held near it impedes progress of the electricity to the conducting body until the electricity accumulates in quantity to overcome the impediment offered in its course, and the electricity escapes in a succession of sparks. This is an illustration of how the current of the nervous principle may be rendered periodic."[*] A well-known application of this explanation to a particular case of nervous rhythm was that given by Rosenthal[†] for the inhibitory action of the superior laryngeal on the respiratory centre (1862). He concluded that the stream of nervous impulses from the constantly discharging and blood-stimulated respiratory centre meets a resistance to its outflow and thereby becomes rhythmic, much as does a stream of air passing out from a tube under water (*op. cit.*, p. 242). He described the function of the superior laryngeal nerve as being to increase this resistance and thus slow and deepen the respiratory movements; and the function of the rest of the vagus as being to lessen the resistance and thus render the respiratory rhythm less deep and more frequent. He argued that other inhibitory nerves act on their respective mechanisms in the same manner as does the superior laryngeal on the respiratory centre.

When a skeletal muscle's reflex contraction is partly antagonised by a reflex inhibition insufficient to suppress it entirely, the contraction thus moderated by inhibition often shows oscillations. A. Forbes[‡] has put the question whether these oscillations may not mean that nerve-impulses constantly generated by the reflex excitatory nerve break through the inhibitory resistance periodically, just as, on Rosenthal's view, do the inspiratory impulses from the respiratory centre.

Undulations tend commonly to appear in reflex contractions obtained under concurrent reflex excitation and inhibition,[§] and both T. Graham Brown[||] and Forbes[¶] have independently called attention to features in them. Of such undulations two main forms may be distinguished, and it seems at present well to regard the two separately, although the same principle of production may underlie both.

In one form, the undulations are smaller, more rapid, less regular, and have a frequency of about 8–10* or 7–12 per second.† Figures showing this form have been furnished in previous papers in these 'Proceedings.'‡ The oscillations are often compounded into or with slower ones so that the graphic records show compound waves.

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[*] John Müller, 'Handb. d. Physiol.,' 1835, vol. 11, p. 77.

[†] 'Die Athembewegungen u. ihre Beziehungen zum Nervus Vagus,' Berlin, 1862.

[‡] 'Roy. Soc. Proc.,' July, 1912, B, vol. 85, p. 289.

[§] 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 268; *cf.* also 'Quart. Journ. Exper. Physiol.,' 1908, vol. 1, p. 7.

[||] 'Roy. Soc. Proc.,' 1912, B, vol. 85, p. 278.

[¶] *Ibid.*

In the other form the undulations are slower and often of much more regular rhythm and amplitude than the above. As seen in figures furnished in these 'Proceedings,' by T. Graham Brown,§ they show on the muscle contraction as notches or teeth in series from three to seven in number, each undulation lasting not far short of a second. Forbes figures‖ a remarkable example with oscillations more ample, less regular, proceeding through a long series with a rate often of about 1 per second.

In a paper of my own an example of this slower form of undulations was figured,¶ recorded simultaneously in flexor and extensor muscles of the knee; it was there (p. 268) pointed out that these undulations are reciprocal in sense in the antagonistic muscles, puffs, so to say, of inhibition in the one muscle's centre corresponding with puffs of excitation in the other's. Graham Brown,** observing the antagonist muscles of the ankle, notes also there that where (fig. 3 of his paper) the undulations were visible in both the antagonists they were reciprocal in them.

An important suggestion is made by Graham Brown, namely, that these slower undulations are "akin to the rhythmic act of progression."†† This suggestion the results given in the present pages leave no room to doubt is correct. Graham Brown believes "that the rhythmic phenomenon is conditioned by a balance of" "activities which produce in the same centre equal and opposite effects (excitation and inhibition)."‡‡ Forbes,§§ referring to the less regular and less orderly oscillations met in his experiments, writes: "Perhaps all" these oscillations "are manifestations of a general tendency of opposed influences in reflex centres, although themselves continuous, to produce rhythmic activity. If so, it is conceivable that some conditions of intensity or time relations produce the regular movements of progression by enabling the centres to fall into a rhythm natural to them, whereas other conditions . . . just miss the natural rhythm of the centres and produce a confused rhythm instead."

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* Forbes, *ibid.*, p. 293.

† *Cf.* 'Quart. Journ. Exper. Physiol.,' *loc. cit.*, p. 74, fig. 6.

‡ Forbes, 'Roy. Soc. Proc.,' B, vol. 85, pp. 293, 297, figs. 3, 4; Sherrington, *ibid.*, B, vol. 80, pp. 569–574, figs. 1–4; *ibid.*, B, vol. 81, pp. 250, 262, figs. 1, 10.

§ 'Roy. Soc. Proc.,' 1912, B, vol. 85, pp. 281–283, figs. 1, 2, 3.

‖ 'Roy. Soc. Proc.,' 1912, B, vol. 85, p. 292, fig. 1.

¶ 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 261, fig. 9, B.

** 'Roy. Soc. Proc.,' *loc. cit.*

†† *Ibid.*

‡‡ *Ibid.*, p. 287.

§§ 'Roy. Soc. Proc.,' *op. cit*, p. 297.

In conformity with this opinion the discrete and regular contractions shown in fig. 1, presenting the appearance of a rhythmic series of wholly separate contractions, which are reciprocals of similar complete relaxations in the fellow muscle, are in reality examples of the above discussed slow form of undulation incident to reflex contraction under concurrent inhibition and

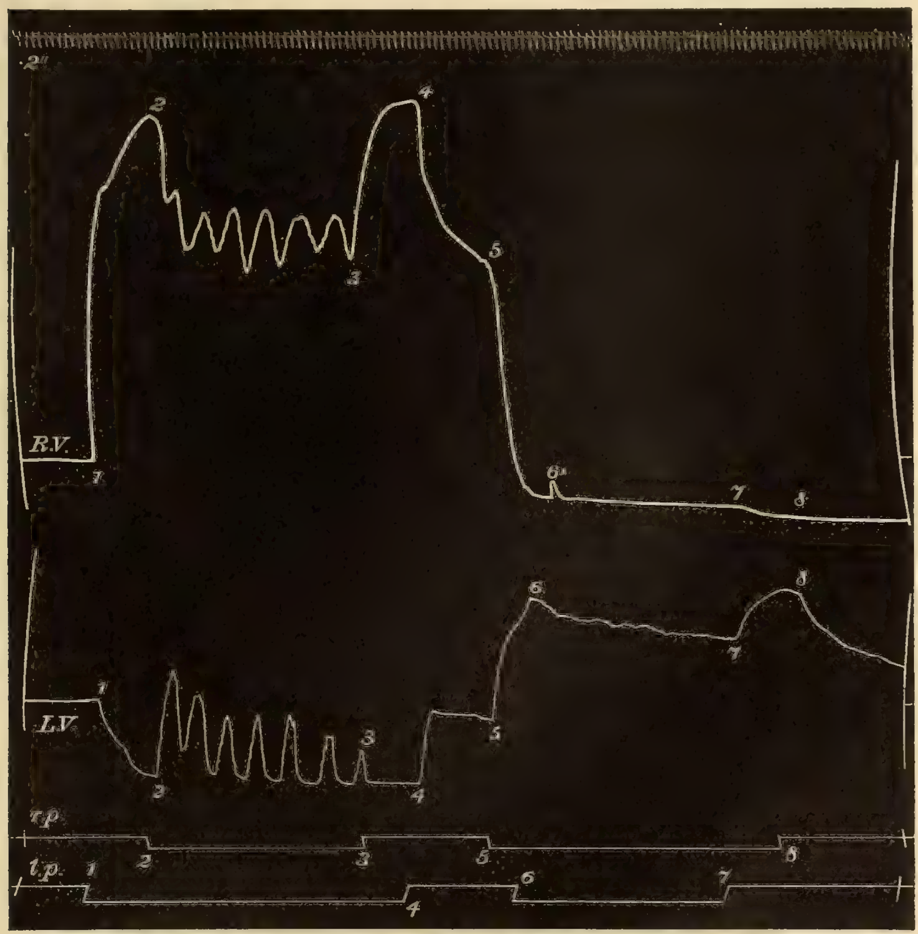

FIG. 2.—Reciprocal stepping of isolated extensor muscle of knees, right and left, reflexly evoked by concurrent stimulation of the antagonistic afferent nerves *l.p.*, left peroneal, and *r.p.*, right peroneal. Decerebrate cat. R.V., right vastocrureus; L.V., left vastocrureus. Correspondences between the stimulation signals and the myograph events indicated by numerals. The right peroneal stimulation following the left produces the rhythmic though somewhat imperfect stepping, but the left following the right, although the same physical stimuli are employed, fails to do so. Time above in fifths of seconds.

excitation; examples, it is true, in which the regularity and amplitude of rhythmic contraction and relaxation have reached their full. That these rhythmic contractions are really analogous to the simple undulatory reflex contractions cited and discussed above seems clear from several considerations. (1) Both occur under the same circumstance, namely, concurrence of inhibition and excitation. (2) Both exhibit the same rate of rhythm. (3) It has not been difficult in my experiments to find intermediate examples connecting the more developed forms with the less developed. Thus, the example fig. 2 is intermediate between the form exhibited by fig. 1, and the example figured in observation A, fig. 6; and the example fig. 3 seems to me intermediate between that of fig. 1 and the example figured by Forbes

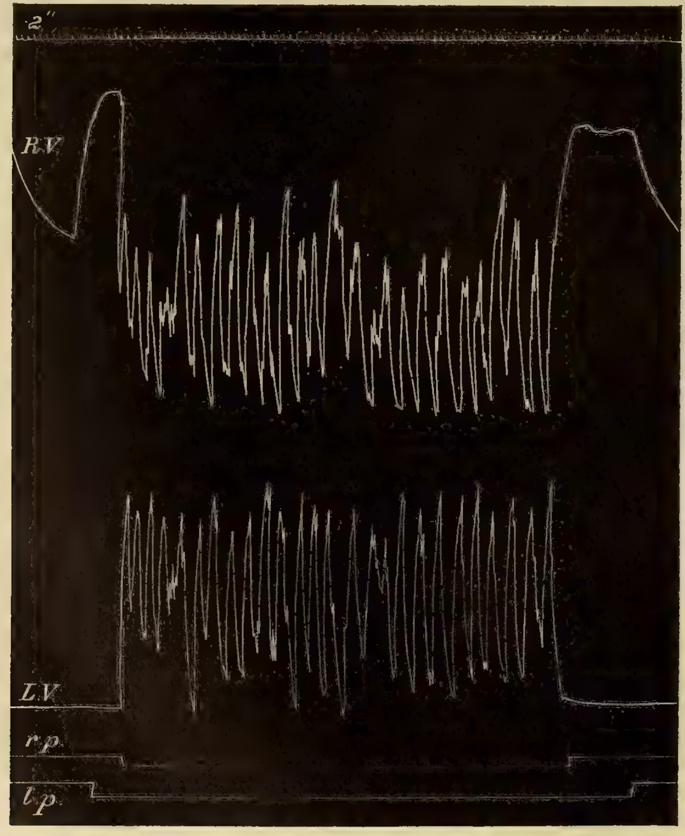

FIG. 3.—Reflex reciprocal stepping of isolated extensor muscle of knees, right and left, evoked by concurrent stimulation of the antagonistic afferents *r.p.*, right peroneal, and *l.p.*, left peroneal. Decerebrate cat. R.V., right vastocrureus muscle; L.V., left fellow muscle. Time above in fifths of seconds. In addition to the rhythmic stepping, which is here somewhat rapid, there is a minute tremor of much more rapid rate (see text) engrafted on and disturbing the stepping reflex's rhythm.

(*op. cit.*, fig. 1). By varying the conditions of experiment various transitional forms can be obtained between imperfect irregular rhythmic movement and the complete regular rhythmic series of movements constituting stepping. Figs. 6, 7, and 15 furnish some illustration of this.

III.

Turning now to analyse some of the features of the rhythmic stepping the example in fig. 1 may be used. The observation begins with stimulation (faradic) of the right peroneal nerve, causing immediate inhibitory reflex relaxation of the right knee extensor with synchronous reflex contraction of the left fellow muscle ; both relaxation and contraction are perfectly steady and arhythmic. After 2·5 secs., during which time the left muscle has remained perfectly steadily relaxed and the right perfectly steadily contracted, stimulation (faradic) of the left peroneal nerve is commenced, that of the right still continuing unchanged. The steady relaxed condition of the right muscle is at once broken by a contraction and at the same moment the steady contracted condition of the left muscle is broken by an inhibitory relaxation. The r. contraction and the l. relaxation culminate synchronously in about 0·4 sec., and then die out about as rapidly as they appeared, to reappear and similarly culminate synchronously again. In this way they rhythmically and reciprocally appear and reappear in series so long as the concurrent stimulation of the two nerves right and left is kept up, namely, for 6·5 secs., seven complete steps being taken in that time by the extensor muscle of each knee. The r. stimulus, the one commenced with, was then withdrawn. The stepping immediately ceases in each muscle, except that the r. muscle, which, having begun its stepping by contraction, is at the end of the seventh step, relaxes, carries out a half-step more and passes into steady contraction, thus assuming an attitude of full extension of knee; and that similarly the l. muscle, which, having begun with relaxation, is at the end of seventh step, contracts, executes a half-step more, passing into relaxation and assuming a posture of flexion of knee. These final half-steps and assumptions of reciprocal states at the two knees are, of course, due to the action of the stimulation of the left nerve now remaining unopposed by any concurrent stimulation of the antagonist right nerve. The l. nerve stimulation remains in operation until withdrawn 3·5 secs. later. During its sole action no trace of rhythm appears in either muscle. On its withdrawal the contraction of right muscle at once begins to decline, although, the preparation being the tonic one, the "shortening reaction" of "plastic tonus" has taken place, and the tonic shortness of the muscle still persists after withdrawal of the stimulus. In the left muscle on withdrawal of left nerve stimulus no obvious change occurs, because the stimulus is inhibitory and under it the muscle is already relaxed and relaxes no further and no less on cessation of the excitation, a "lengthening reaction" having occurred at end of the r. nerve stimulation.

Three seconds later the left nerve stimulation is recommenced; steady contraction of r. muscle is reassumed; l. muscle being already relaxed the inhibitory effect there is not apparent, although really fully existent, as subsequent events show. The l. nerve stimulus is here the pre-current stimulus, and under it no rhythm of any kind appears in either muscle any more than did under r. nerve stimulus in the preceding observation. But on then applying stimulation of r. nerve, that of l. still continuing as before, rhythmic stepping at once appears. It begins synchronously in right and left muscles, the opening phase being contraction, *i.e.* extension of limb, in the left muscle, and relaxation, *i.e.* flexion of limb, in the right muscle. On withdrawing the r. nerve stimulus, about 6 secs. later, when the last step of a sequence of six has been nearly completed, the stepping ceases abruptly with completion of that step. The right muscle then reverts to steady contraction, the left to full relaxation. This resumption is, of course, the effect of the still-continuing l. nerve stimulation, and guarantees that the stimulation of that nerve has remained effective throughout. Rather more than a couple of seconds later the l. nerve stimulation is withdrawn and the contraction of right muscle, in consequence, shows decline, modified, however, by the shortening reaction of the plastic tonus.

Two seconds later r. nerve's stimulation is once more commenced. It evokes steady contraction of l. muscle and, synchronously with that, full inhibitory relaxation of r. muscle. During the 3·5 secs. for which this stimulus remains thus in operation by itself, the steady reciprocal reaction of the two muscles is maintained unchanged. The stimulation of l. nerve is then commenced, that of r. nerve continuing unaltered. Synchronous stepping, reciprocal in direction in the two muscles, sets in at once. Here the opening phase in each muscle is the reverse of that in the previous observation. The l. nerve stimulus is maintained in concurrence with that of r. nerve for 7 secs., and during that period a sequence of six complete and regular steps is performed by each muscle. Stimulation of l. nerve is then withdrawn, and both muscles at once revert to the non-rhythmic steady state they exhibited previously under stimulation of r. nerve alone, r. muscle being in steady relaxed condition, and l. muscle held steadily contracted. Later, the stimulation of r. nerve is itself finally withdrawn; the contraction of l. muscle then at once begins to decline into pure statotonus, and r. muscle shows a slight return to greater tone than it had during the influence of r. nerve's stimulation.

Although the results, therefore, appear curiously simple, the conditions underlying them are a complex of various factors. Without pretending to offer any full analysis of these, some of them may be here briefly adverted to.

1. *Influence of the Laterality of the Stimulus.*

Decisive points here are (1) that reciprocal innervation holds for the symmetrical muscles, (2) that with extensors the direction of reciprocity is excitation contralateral and inhibition ipsilateral, with flexors conversely, and (3) that the rhythmic stepping here concerned arises, proceeds, and ceases with commencement, continuance, and withdrawal of the exercise of double reciprocal innervation.

In such observations as shown in figs. 1, 4, and 9, we may term the stimuli according to their sequence "pre-current," "added," and "remaining." It will be seen from the figures that the added stimulus when extensor muscles are used always causes, as its first effect, the contraction phase of the contralateral muscle's reaction, and the relaxation phase of the ipsilateral muscle's reaction. That is, it causes, as its first effect, the extension phase of the step at the contralateral knee and the flexion phase of the step at the ipsilateral knee. This is in agreement with the rule observed in the case of stepping provoked by direct faradisation of the spinal cord. As noted previously,* I found that regular stepping of the hind limbs is readily provoked in the spinal animal by unipolar faradisation applied with a stigmatic electrode directly to a certain area of the distal cut face of the cord in the cervical region. If the electrode be applied in the right lateral half of the cord the stepping begins with flexion phase of right hind limb, and extension phase of step in left hind limb.

It might have been supposed that the reflex stepping produced by the double reciprocal action of symmetrical r. and l. afferents upon the knees r. and l. symmetrical extensor muscles must of necessity be bilateral. Experiment shows, however, that that is, in fact, not the case. The reflex stepping so obtained, although usually bilateral, is sometimes unilateral. Thus, figs. 4 and 5 are from the same experiment at short interval. In the former the stepping is bilateral, in the latter it is confined to the left muscle. The observation in fig. 4 was obtained with values of stimuli right nerve 15 cm. left nerve 17 cm. as measured on scale distances of the two induction apparatus.

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* 'Journ. Physiol.,' 1910, vol. 40, p. 86; 'Brain,' 1910, vol. 33, p. 13; also Roaf and Sherrington, 'Quart. Journ. Exper. Physiol.,' vol. 3, p. 210; and G. Brown and Sherrington, *ibid.*, vol. 4, p. 202.

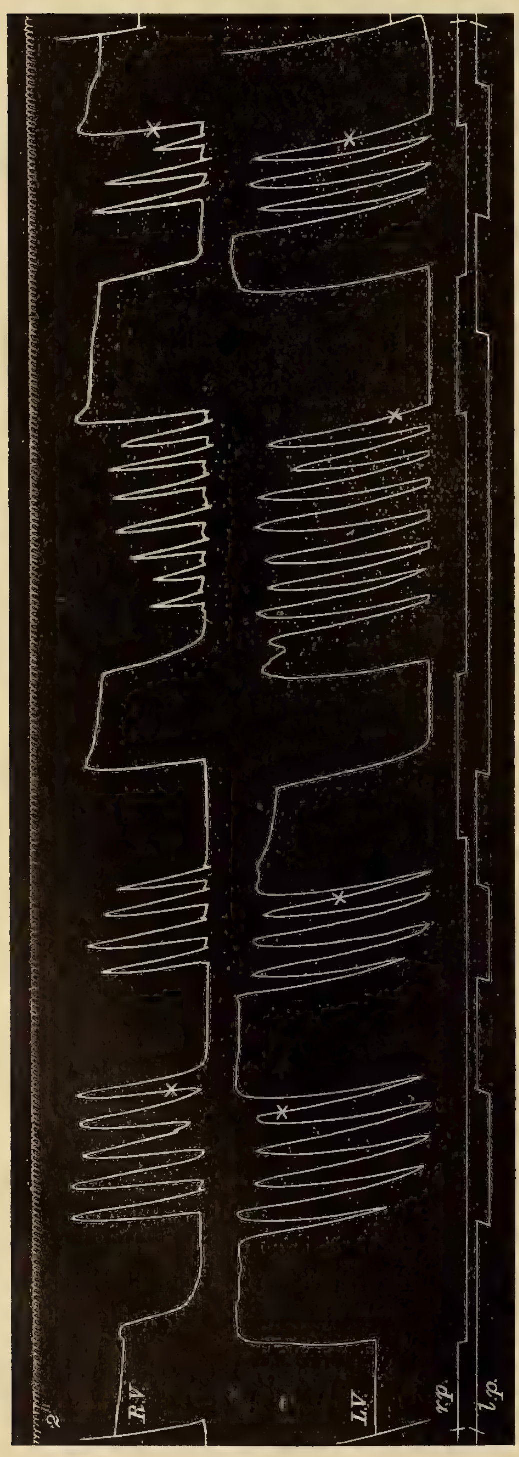

Fig. 4.—Reflex reciprocal stepping of isolated extensor muscle of knees, right and left, evoked by concurrent stimulation (faradisation) of the antagonistic afferents *r.p.*, right peroneal, and *l.p.*, left peroneal. R.V., right vastocrureus muscle; L.V., left vastocrureus muscle. Time above in fifths of seconds. Crosses mark moment of withdrawal of the concurrent stimulus. Stimulus values $\frac{\text{r.p. 15 cm.}}{\text{l.p. 17 cm.}}$. Further description in text, p. 243. From same experiment as fig. 5.

The threshold stimulus for r. nerve had been found slightly higher than that for left in this preparation; the two stimuli although not equal (as shown later *v. infra*) were certainly not far from equal. Then, for obtaining observation, fig. 5, the r. nerve stimulus was increased to 13 cm. and l. nerve stimulus to 16 cm. Both stimuli were therefore increased, but that of r. nerve much more than that of l. nerve, the steepness of ascending intensity of the physical stimulus being much greater between 15 cm. and 13 cm. than between 17 cm and 16 cm. of the scale. This unequal increase of intensity of stimulation of the two nerves r. and l. is answerable for the change from bilateral stepping to unilateral. It suppresses the stepping in the muscle on the same side as the stronger stimulus, and at the same time makes the stepping of the other, the left, muscle faster than it was in fig. 4. It brings this about in the following way. With increase of the strength of the stimulus its ipsilateral inhibition increases more than does its contralateral excitatory effect. When (fig. 4) r. stimulus has a value of 15 cm. it does not suppress the contralateral excitatory effects of l. stimulus at value 17 cm., but

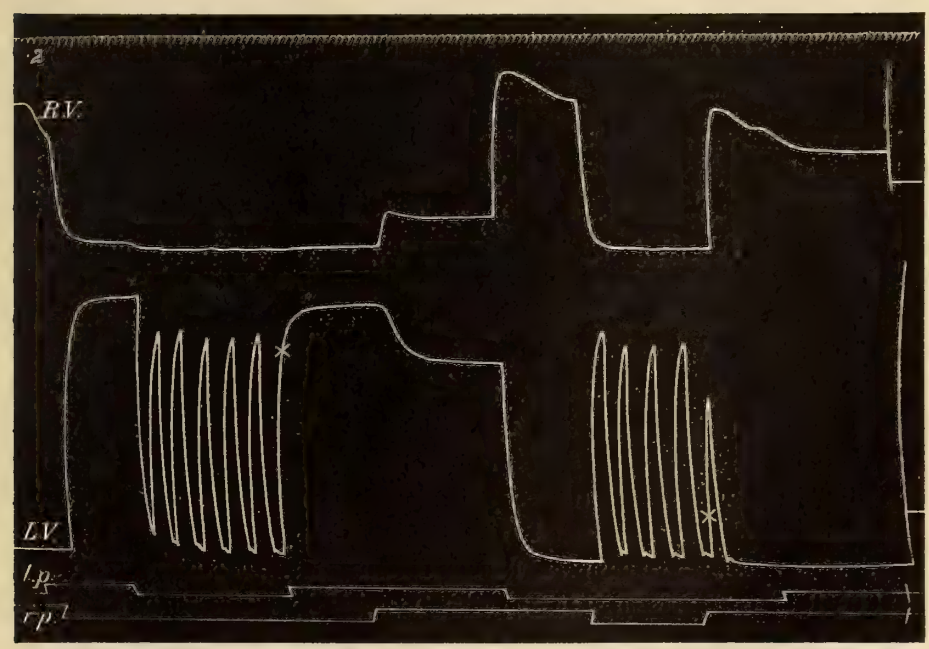

Fig. 5.—Reflex unilateral stepping as exhibited in an isolated extensor muscle of left knee, the similarly isolated extensor of right knee being relaxed by inhibition while the fellow muscle steps. Decerebrate cat. From same experiment as fig. 4, but with different combined stimulus value, namely $\frac{\text{r.p. 13 cm.}}{\text{l.p. 16 cm.}}$. *r.p.* and *l.p.*, right and left peroneal nerves. Time above in fifths of seconds. Further explanation in text, p. 245.

r. at value 13 does suppress all contralateral excitatory effects from l. at value 16. The r. muscle therefore in fig. 5 does not step. But in the l. muscle l.'s stimulus value 16 restrains the contralateral excitatory effects of r.'s stimulus value 13 even less than was the case when l.'s stimulus was value 17 and r's stimulus value was 16. Hence the l. muscle steps faster in fig. 5 than in fig. 4, r.'s excitatory effect breaking through l.'s inhibition more rapidly and frequently. This quickening of the step under the stronger stimulus harmonises with the observation that though the direct faradisation required to excite stepping from a point in the cross-section of the cervical spinal cord is of quite weak intensity, the rate of the stepping of hind limb so produced increases, *ceteris paribus*, with the intensity of the faradic stimulus.

2. *Strength of Stimulus.*

As to the strength of the antagonistic stimuli which by their concurrence evoke the stepping, the phenomenon, in my experience, is not obtainable with strong stimuli. With stimuli just above threshold intensity I have at times seen traces of the rhythmic undulation; but attempts to develop it with such very weak stimuli have not so far attained much result. Stimuli rather stronger than such but on the weak side of moderate have yielded the best results. Weakening the stimuli beyond that point gives a rhythm not only slower but more irregular with waves of varying amplitude (fig. 6A), and a tendency to pauses between some of the beats. On the other hand, with too strong stimuli there is a tendency for the rhythmic reaction to be suppressed in one or other member of the muscle-pair, and for it to be represented in the other member by a few sharp somewhat irregularly explosive beats separated by longish unequal intervals (fig. 6B). An idea of the range of intensities suitable may be afforded by the data of an experiment. In the experiment from which figs. 6A and 6B, and also fig. 4, have been taken, the threshold value for reflex effect both ipsilateral and contralateral lay for r. nerve at 17·8 cm. and for l. nerve at 19·2 cm. This seems high, but it must be remembered that a resistance box of 100,000 ohms was included in each circuit. Slight but distinct rhythmic stepping was obtained by the combination r. at 16 cm. and l. at 18 cm. $\left(=\frac{\text{r. }16}{\text{l. }18}\right)$, fig. 6A. More regular and stronger rhythm was obtained with various stronger combinations, *e.g.*, $\frac{\text{r. }15}{\text{l. }17}$ (fig. 4), $\frac{\text{r. }14}{\text{l. }17}$, $\frac{\text{r. }13}{\text{l. }15}$. Results with $\frac{\text{r. }12·5}{\text{l. }13·5}$ were less good. A result with $\frac{\text{r. }11}{\text{l. }12·5}$ is given in fig. 6B; it exhibits no stepping with right muscle, and merely two short unequal steps with left muscle in a period of concurrence of the stimuli covering more than 3 secs. With $\frac{\text{r. }10{\cdot}5}{\text{l. }12}$, and all stronger combinations tried, there was obtained no rhythmic stepping movement at all, although excellent steady contractions and inhibitions.

Weaker stimuli tend, in my experience, to give slower rhythm. Other factors in the production of the rhythm, for instance, the ratio between the strengths of the antagonistic stimuli, change, however, of necessity also in such comparisons. Still my facts, as far as they at present go, clearly indicate the above tendency. Thus figs. 4 and 6 are from the same experiment. In fig. 6A the stimuli were $\frac{\text{r. }16}{\text{l. }18}$, and the beats are 9 during 8·5 secs., while in fig. 4, observation 3, where the stimuli were $\frac{\text{r. }14}{\text{l. }17}$, the beats are 7 during 5·2 secs. Again fig. 7, observations A, B, and C are all

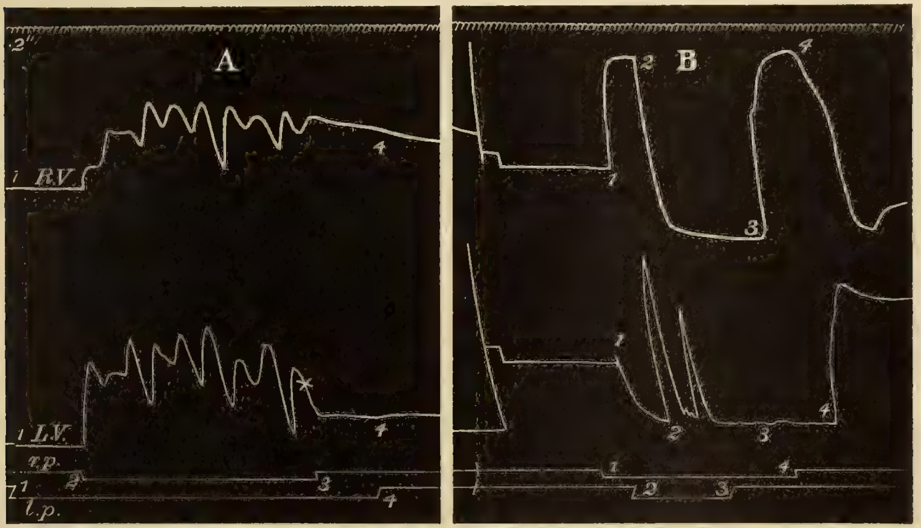

Fig. 6.—Reflex rhythm (imperfect stepping) evoked in the isolated extensor of knees, right and left, by concurrent stimulation of the antagonistic afferents, right and left peroneals, *r.p.* and *l.p.* Decerebrate cat. In Observation A the stimulus values are too weak $\frac{\text{r.p. }16\text{ cm.}}{\text{l.p. }18\text{ cm.}}$; in Observation B they are too strong $\frac{\text{r.p. }11\text{ cm.}}{\text{l.p. }12{\cdot}5\text{ cm.}}$; both observations are from the same experiment as fig. 4, where good and regular reflex stepping is obtained with stimulus values $\frac{\text{r.p. }15\text{ cm.}}{\text{l.p. }17\text{ cm.}}$. In Observation A it is noteworthy that the stimulation of *l.p.* is so weak that it of itself produces no obvious contraction of the contralateral muscle and only the merest trace of relaxation of the ipsilateral muscle. Yet its presence is documented at once when the concurrent stimulus is added in the response being then not steady but rhythmic. Further explanation in text, p. 246.

from one experiment. In fig. 7A the combined stimuli $\frac{\text{r. } 14}{\text{l. } 16}$ give five steps in 14 secs., but strengthening stimulus r. to 13, so that the combined stimuli become $\frac{\text{r. } 13}{\text{l. } 16}$, results (fig. 7B) in 13 steps during 17·5 secs., and strengthening both stimuli further still to $\frac{\text{r. } 12}{\text{l. } 13{\cdot}5}$ produces (fig. 7C) five steps during 3·8 secs.

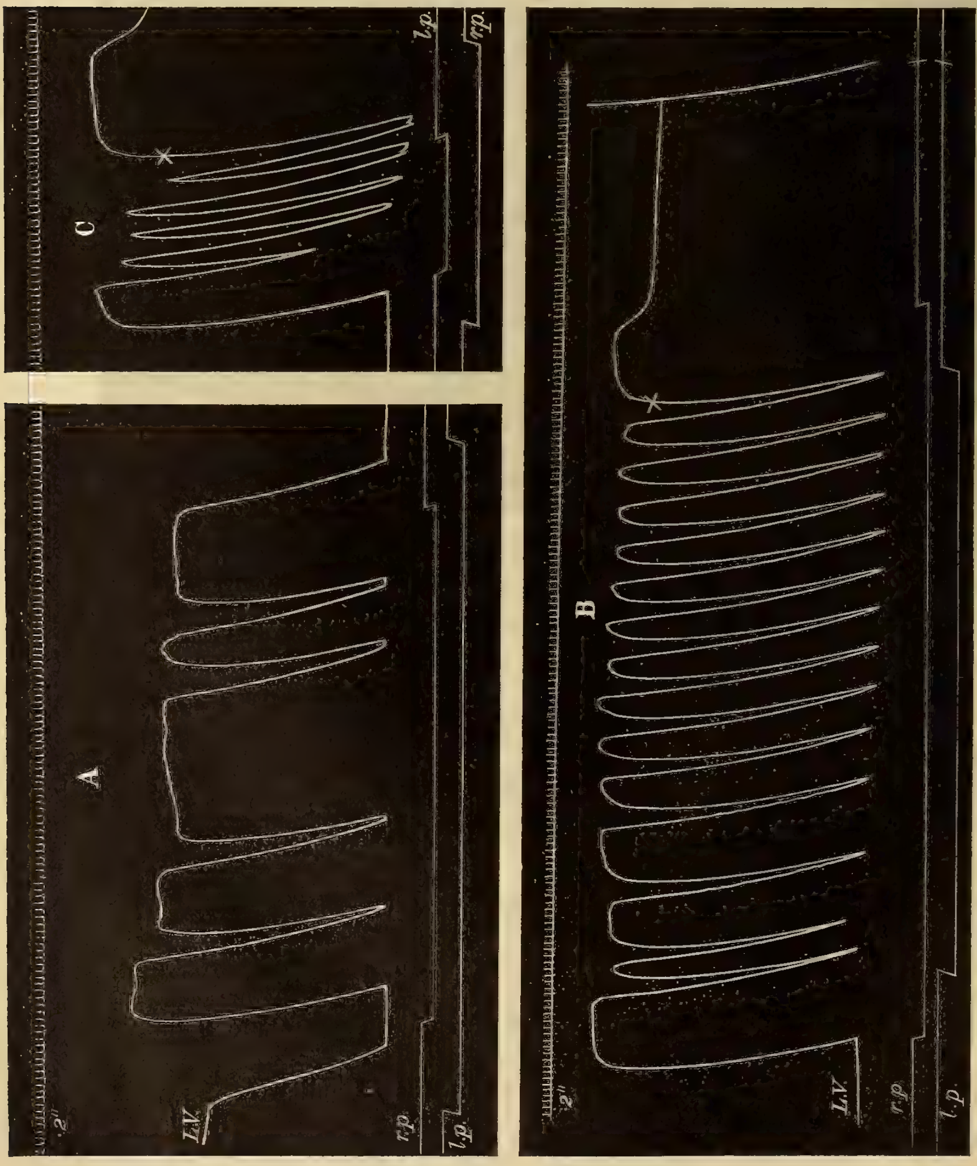

FIG. 7.—Reflex rhythmic stepping of isolated extensor muscle L.V. of left knee (decerebrate cat) evoked by concurrent stimulation of *r.p.*, right peroneal, and *l.p.*, left peroneal, nerves as afferents. Stimulus values for Observation A are $\frac{\text{r.p. } 14 \text{ cm.}}{\text{l.p. } 16 \text{ cm.}}$, for B are $\frac{\text{r.p. } 13 \text{ cm.}}{\text{l.p. } 16 \text{ cm.}}$, and for C are $\frac{\text{r.p. } 12 \text{ cm.}}{\text{l.p. } 13{\cdot}5 \text{ cm.}}$. Time above in fifths of seconds. Further explanation in text, p. 247.

More satisfactory for comparison is fig. 8 with fig. 5. Both are observations from the same experiment, with no long interval between them. The l. stimulus was of the same value in both, namely, 16 cm. But the r. stimulus had value 15·5 cm. in the observation fig. 8, whereas in that of fig. 5 its value was 13 cm. The results contrast in three striking features. Where r. stimulus is stronger (fig. 5) the rate of stepping is six double phases in 4·8 secs., as against four double phases in 4 secs., where r. stimulus is weaker. Where r. stimulus is weaker (fig. 8) the stepping is not so well maintained as where r. stimulus is stronger. Where r. stimulus is weaker (fig. 8) the stepping occurs in the fellow muscle of the right side as well as in the left, and where r. stimulus is stronger (fig. 5) the stepping is confined to the left-side muscle.

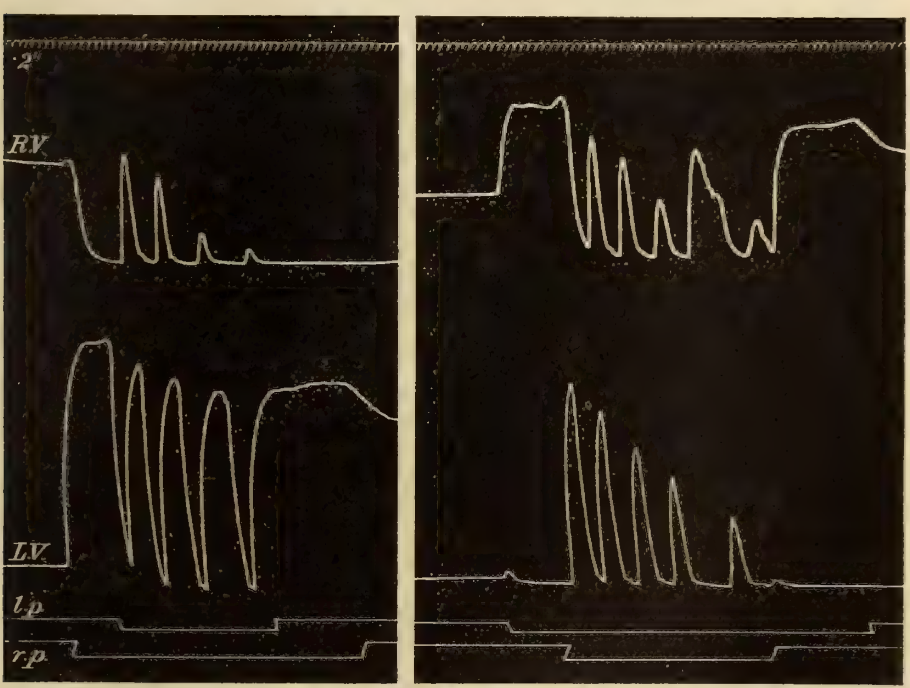

Fig. 8.—Reflex reciprocal stepping as exhibited by isolated extensor muscle of knees, right and left, evoked by concurrent stimulation of antagonistic afferents, right and left peroneals, *r.p.* and *l.p.* Decerebrate cat. Time above in fifths of seconds. Compare fig. 5. Explanation in text, p. 249. Stimulus value $\frac{\text{r.p. } 15\cdot5 \text{ cm.}}{\text{l.p. } 16 \text{ cm.}}$.

3. *Influence of Intensity-ratio between the two Antagonistic Stimuli.*

From the preceding section it is clear that as might be expected the proportion between the intensities of the two antagonist stimuli is an important factor in determining the existence and characters of the rhythmic reflex. Evidence of this was constantly met in the experiments. In fig. 7 the two observations A and B illustrate an aspect of it. Stepping is more marked in B than in A although the intensity of one only of the rival stimuli was altered. Conversely, the change of intensity by even a little of one of the antagonist stimuli may make all the difference to whether the combination be effective or not for rhythmic stepping. My experience put generally is

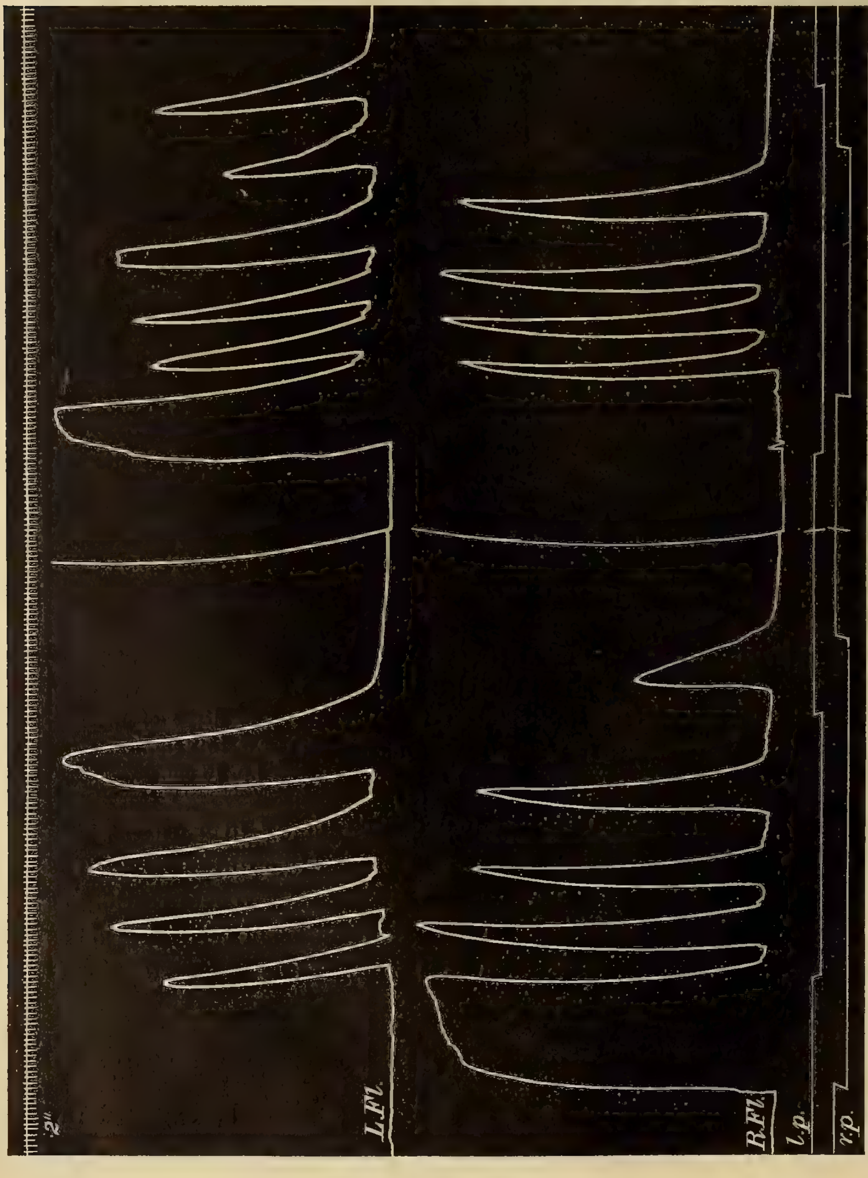

Fig. 9.—Reflex reciprocal stepping as exhibited by isolated flexor muscles, R.F., L.F., of hips, right and left, evoked by concurrent stimulation of right and left peroneal nerves, *r.p.* and *l.p.* The direction of the reciprocal innervation is here contraction ipsilateral and inhibitory relaxation contralateral. Time above in fifths of seconds. Decerebrate cat. The muscles, both right and left, were psoas and tensor fasciæ femoris.

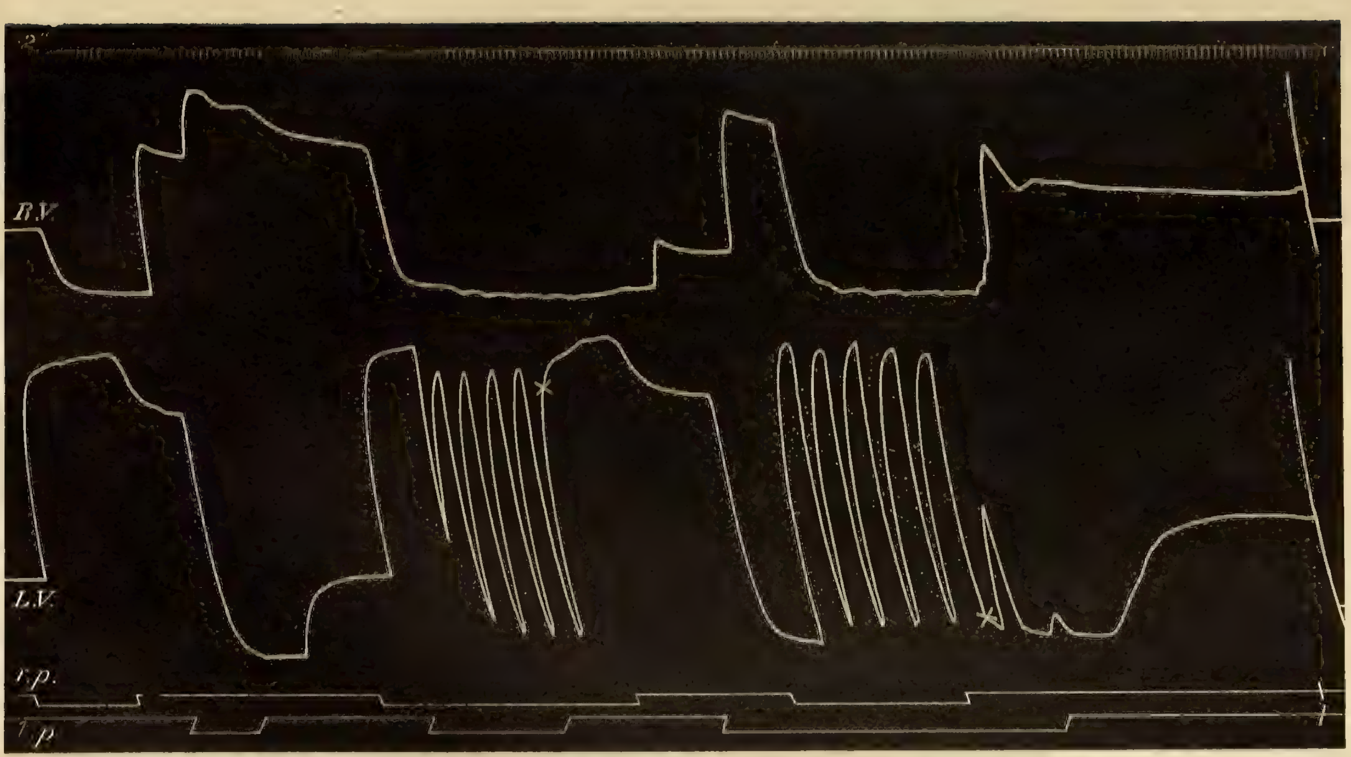

FIG. 10.—Reflex stepping induced in the isolated extensor muscle of the left knee, but not in that of the right, by concurrent stimulation of the antagonistic afferent nerves, *r.p.*, right peroneal, and *l.p.*, left peroneal. Decerebrate cat. R.V., right vastocrureus muscle; L.V., left vastocrureus muscle. Time above in fifths of seconds. Stimulus values $\frac{\text{r.p. 13 cm.}}{\text{l.p. 16 cm.}}$. The first two observations in the figure show the separate reactions of the two separate stimuli which, when concurrent, give the unilateral stepping of the two last observations of the figure. Further explanation in text, p. 252.

that the antagonist stimuli must not be very unequal in intensity if they are to provoke the rhythmic reflex (fig. 9 shows this with flexor muscles), but that on the other hand the stimuli may yet succeed in producing the rhythmic reflex when quite indubitably and markedly unequal in intensity, if that inequality does not go beyond certain limits.

Fig. 10 will serve as illustration. In this experiment the thresholds of r. and l. nerves lay at 19·5 and 20·5 respectively. The physical circuits were hardly appreciably unequal, for the thresholds changed only to 19·2 and 20·8 when the coils were interchanged for the two nerves by the double

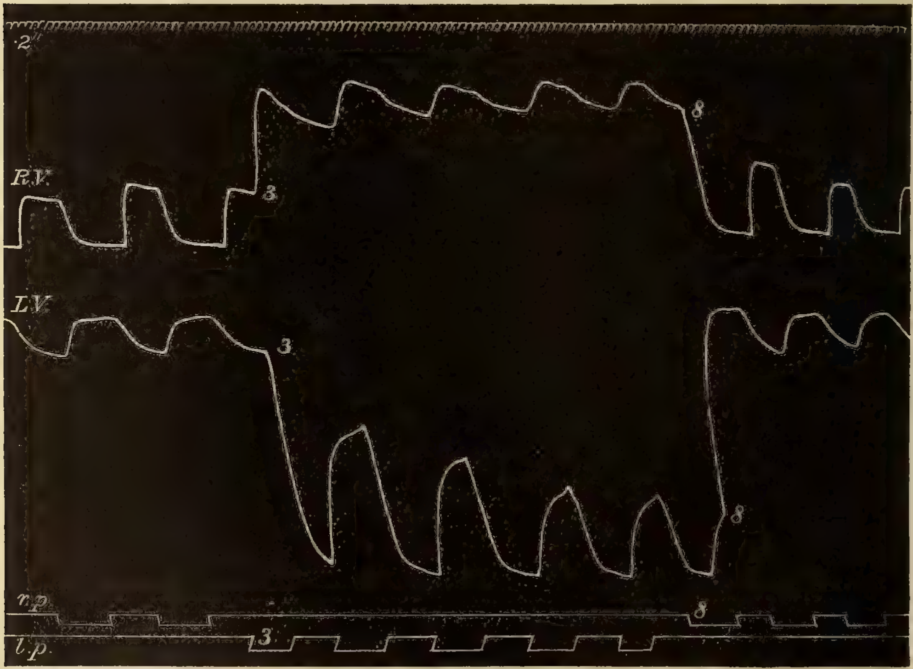

Fig. 11.—A series of reflexes, phasic and tonic, provoked in the isolated right and left vastocrureus muscles by short non-concurrent faradisations of right and left peroneal nerves. The numerals mark some salient corresponding events in the signal lines and myograms. The inhibitory relaxations produced in right vastocrureus, R.V., by stimulation of *r.p.* are followed by rebound contractions; similarly the inhibitions of L.V. are followed by post-inhibitory rebounds on withdrawal of each stimulation of *l.p.*, left peroneal. Besides the reciprocal reflex movements induced by each stimulus, the stimuli cause assumption of reflex postures, *e.g.* under stimulation of *r.p.* the two myogram lines approach each other, *i.e.* r. muscle is relaxed (indicating that flexion of right leg would be going on, were the muscles not paralysed) and l. muscle is contracted (indicating that left limb would be extended). Under stimulation of *l.p.* the two myograph levers diverge, indicating that left limb assumes posture of flexion and right limb that of extension. This figure gives the separate effects of the two stimuli which, when concurrent, give the stepping reflex recorded in fig. 1. Decerebrate cat. Time in fifths of seconds.

switch. This being so we must consider the stimuli r. 13 and l. 16 distinctly unequal. Their separate effects on the preparation are shown at the beginning of fig. 10. It is noticeable there that the rebound contraction after the inhibition produced by r. 13 is greater than after that produced by l. 16; also that r.'s contractive effect on L muscle is better maintained than is l.'s on R muscle. Yet this combination produces, as the figure shows, good stepping in one of the muscles. Now this fig. 10 is from the same experiment as furnished fig. 1 and the combined stimuli in fig. 1 are $\frac{\text{r. }14}{\text{l. }17}$. And $\frac{\text{r. }13}{\text{l. }16}$ must be considered less equal than $\frac{\text{r. }14}{\text{l. }17}$, yet the rhythmic stepping of the left muscle in fig. 10 is as good or better than that in fig. 1. Now the stimuli used in fig. 1 were themselves unequal, not merely on the face of their scale values but as tested on the preparation at the time. Fig. 11 shows the effects of these two stimuli when employed separately; the tonic effect is obviously not equal but also not far from equal; but (fig. 12) the rebound is greater after inhibition

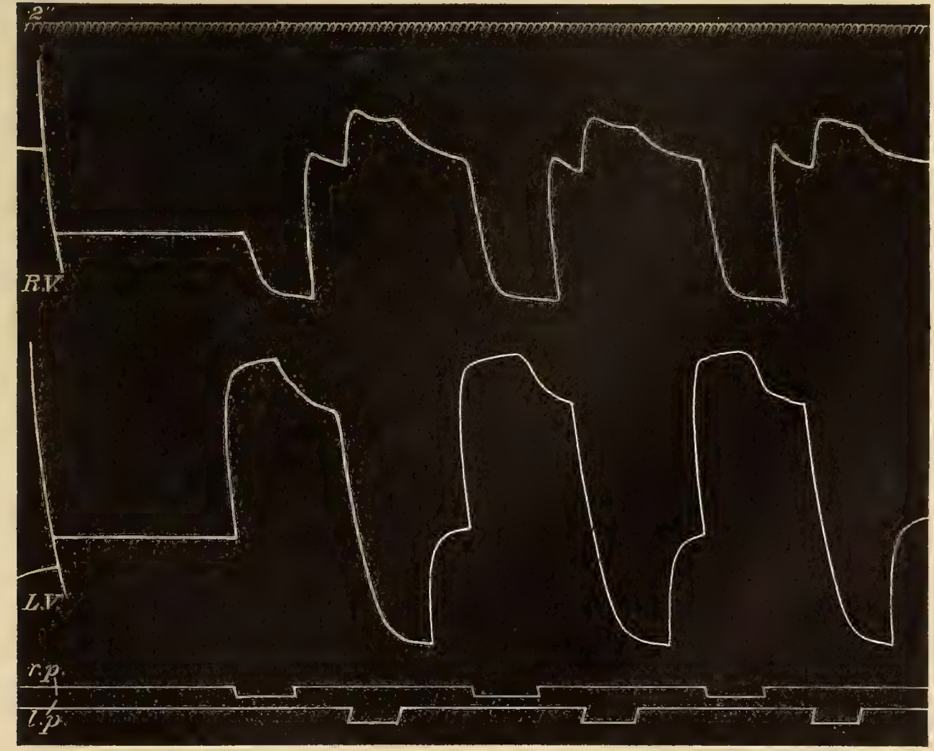

Fig. 12.—Right knee extensor (R.V.) and left (L.V.) isolated and reacting reciprocally to reflex stimulation of each peroneal afferent; *r.p.*, right peroneal, *l.p.*, left peroneal. Time above in fifths of seconds. Decerebrate cat.

by r. 14 than after inhibition by l. 17. And when, in fig. 13, the r. and l. stimuli are applied synchronously (observation 50) and begin together, r. stimulus obviously has the upper hand, for L muscle at once contracts, and though not seen in the figure R muscle at once relaxed. Moreover on withdrawing the two stimuli together L muscle at once relaxes instead of showing rebound contraction. Clearly r. stimulus is stronger than l. stimulus in its effect on the preparation. Observations 50, 51, 52, 54, fig. 12, in which r. and l. stimuli are applied together, do not differ in result on the muscle from observations 48 and 49, fig. 12, in which r. stimulus was applied alone, except in the feature that some little while after commencement of each of the double stimulations (observations 50–54) an inhibitory notch appears in the reflex contraction, whereas in observations 48 and 49 there is nothing of the kind. Reserving this point of difference for the present, the result here is that of the two stimuli r. 14 and l. 17 the former is the stronger not only in its numerical scale-value but also as actually tested on the preparation. Yet this pair of unequal stimuli give a good rhythmic reflex during their concurrent application (figs. 1 and 13). If r. 14 and l. 17 form an unequal pair in which r. is the stronger, obviously r. 13 and l. 16 must form a pair more unequal still. Yet $\frac{\text{r. } 13}{\text{l. } 16}$ gives a good rhythmic reflex in one of the muscles (fig. 13).

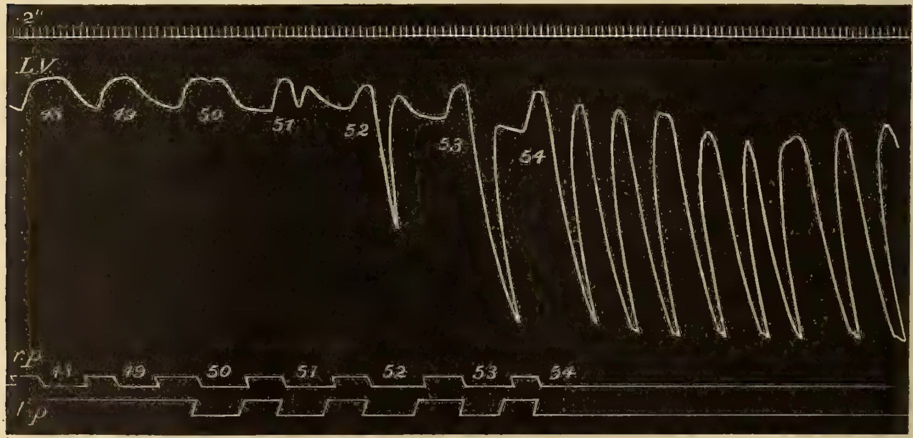

Fig. 13.—Extensor muscle of left knee L.V. Observations 49 and 50 show reflex contractions evoked by stimulation of right peroneal nerve, *r.p.* Then 50–54, a series of synchronous stimulations of *r.p.* and *l.p.*; during these there ensues stepping; the inhibitory phase (flexion phase) of the step appears as a gradually increasing inhibitory notch on the contraction caused by *r.p.* Time above in fifths of seconds.

Certainly, therefore, in order to produce the rhythmic reflex, the two antagonistic stimuli, right and left, need not be exactly equally balanced but experience points to the necessity of their being not widely unequal, also to the need for closer balance of r. and l. stimuli for evoking bilateral rhythmic reflex than for evoking a unilateral one.

Results referred to at the outset of this communication throw some light on this. It was there said that as the intensity of stimulus used for evoking the reciprocal reflex on the muscle pair is progressively increased, the intensity of both ipsilateral inhibition and contralateral contraction increase, but that of the former more rapidly than that of the latter. Synchronous application of r. and l. stimuli of equal strength produces when the stimuli are strong suppression of contraction both r. and l.; but with weak stimuli bilateral contraction, contraction of both muscles, results. The same result is shown by determining the strength of contralateral stimulus required to force its reflex contraction through an already established ipsilateral inhibition. The kind of result then met with is as follows: A preparation where threshold for r. peroneal was 16·4 cm., and that for l. peroneal 15·2 cm., yielded the figures—

	cm.		cm.
Inhibition of R. muscle by r. stim.	13	was broken by reflex contract. due to l. stim.	14
„ „	12	„ „ „	12·5
„ „	11	required l. 10 cm. to break it through.	
„ „	10·5	„ l. 9 „ „	

Such results indicate a relation between intensity of stimulus and intensities of ipsilateral and contralateral reflexes such as is sketched in the diagram. The diagram accounts for the relation between the observations of

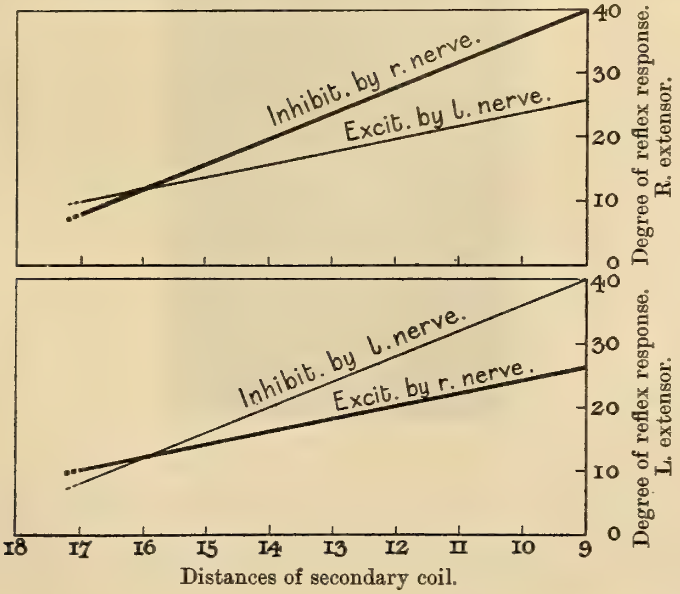

fig. 4 and fig. 5, both from the same experiment; also for that between the observations of fig. 1 and fig. 10, both from the same experiment. As drawn, the diagram applies to extensors (of knee), but by reversing inhibition to excitation and conversely it is applicable to flexors (of hip and knee).

4. *Influence of the Sequence.*

It is noticeable that the effect of concurrence of the stimuli opens with a result in the direction of that of the added stimulus practically at once on addition of that stimulus. Under the concurrence the newer stimulus tends to dominate at once. This is in harmony with experience on visual rivalry, struggle between rival contours, etc. The very newness of the new stimulus lends it force as against the pre-existent, especially where the pre-existent is not very much the more potent and has been in operation for some time.

Probably a similar relation explains why although well-acting combinations of the antagonist stimuli produce the rhythmic reflex whichever of the two stimuli precedes, with less effective pairs of stimuli that is not the case. It appears with the latter a point material for the result which of the two precedes and which follows (fig. 2). The rhythmic reflex may be much better if x precede y than *vice versâ*, or it may not appear at all in one sequence

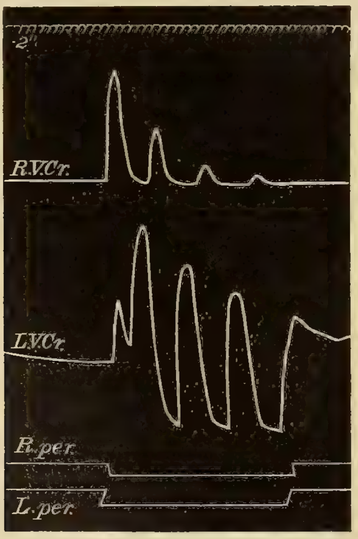

FIG. 14.—Isolated extensor muscle of right R.V. and left L.V. knees. ynchronous stimulation of both right and left peroneal afferents (*R. per.*), (*L. per.*) The reflex opens with identical contraction of both the muscles, but almost immediately the reaction of the left muscle is changed to inhibitory relaxation, with the result that the symmetrical muscles fall into step with reciprocal harmony. Decerebrate cat. Time above in fifths of seconds.

though appearing with the other. A particular combination which works need not necessarily work reversed, though it generally does so.

As to what happens when the two rival stimuli are started together, the observations have shown that sometimes then the reflex step starts at once with full reciprocity of phase in the two muscles, and so proceeds co-ordinately in that manner. Sometimes, however (fig. 14), the muscular effect begins identically with contraction in the two muscles, and this is almost immediately checked and reversed in one of the muscles so that that muscle then begins, although a little late, to behave reciprocally in regard to its fellow. In fig. 14 this correction takes place in time for L. muscle, although starting wrongly, to have got into harmonious reciprocal step with its fellow before even the completion of the first phase of the first step is reached Harmonious reciprocity then continues.

When the two rival stimuli are started together there seems a tendency for the stronger to overcome the weaker altogether at first, and then to give way to the latter a little later, and then later still to re-establish ascendancy, again soon losing it, and so on. Thus a see-saw alternation of dominance is arrived at. Observation 54 in fig. 13 exemplifies this. The weaker stimulus (*v. supra*) l. 17 there makes its effect felt as a deep inhibition of a contraction already initiated by r. 14. And in the series of immediately precedent observations 50—53, this effect of l. 17 is seen to have each time occurred with the same time-relations, although with successively increasing effect. Evidence in the same direction is illustrated in fig. 7A.

5. *Influence of the Afferent Nerve of the Reacting Muscle.*

The rhythmic effect of the antagonistic concurrent stimuli takes place when the reacting muscle has been de-afferented. The presence of the proprioceptive afferents of the muscle is therefore not necessary to the reaction. Fig. 15 shows the rhythmic stepping obtained from the right vastocrureus by concurrent stimulation of the antagonistic r. and l. popliteal nerves. (The signals in this experiment were set to mark upwards, not downwards as in the other figures of this paper.) The observation begins with faradisation of r. nerve producing, after the initial spike (*Anfangstetanus*), inhibition; but the muscle being already relaxed, the preparation being decapitate, the inhibition causes no visible further elongation of its muscle, except the suppression of the initial contraction which it itself had provoked. After 2·5 secs. the stimulation of the contralateral popliteal is commenced, the stimulation of ipsilateral proceeding unaltered. The result of concurrence of the stimuli is immediate rhythmic stepping of the muscle. Four steps are taken during the continuance of the concurrent

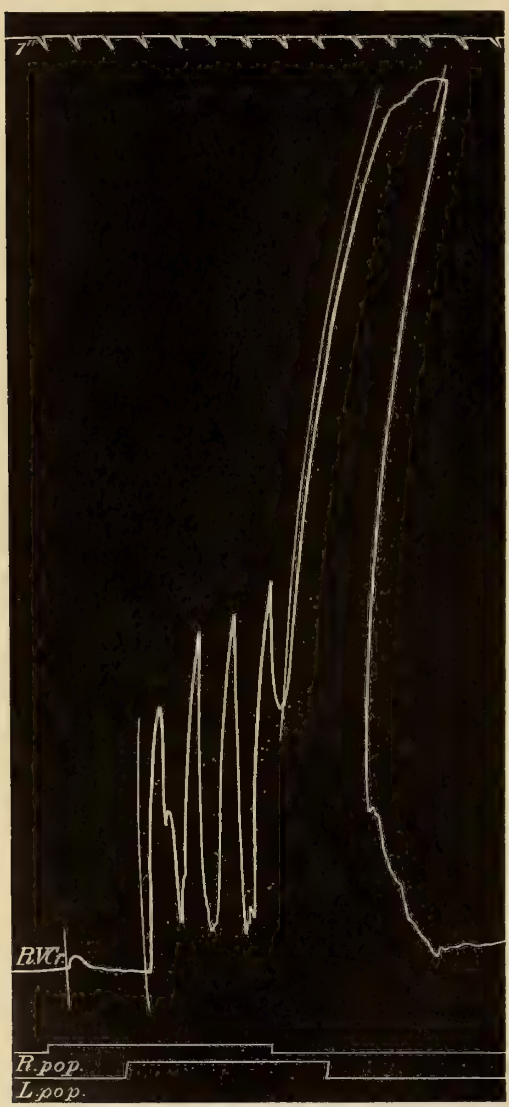

Fig. 15.—Isolated de-afferented extensor muscle of right knee. Decapitate cat. Faradisation of central end of right popliteal nerve for 6·5 secs., joined 2·2 secs. after its commencement by faradisation of left popliteal nerve; the latter stimulation is continued until 2 secs. after cessation of right popliteal stimulation. The signal marks are directed upward instead of downward as in the other records. During the concurrence of the stimuli, but not when either of the two stimuli is in operation alone, the muscle gives rhythmic reflex stepping; it completes four steps in about 4 secs. The muscle had been de-afferented (118 days). The proprioceptive reflex—"shortening reaction"—is therefore absent on withdrawal of the contralateral stimulus, and the muscle relaxes at once, being toneless. Time above in seconds.

stimulation, *i.e.*, in 4·5 secs.; the ipsilateral stimulus is then withdrawn, and under the influence of the remaining contralateral stimulus the muscle enters at once into steady maintained contraction, and continues so contracted until the stimulus is withdrawn. The muscle had been de-afferented October 7, 1908, and was used for experiment nearly four months later, February 2, 1909. In the experiments of Graham Brown* it has been shown that stepping occurs after de-afferenting the muscles involved.

6. *Influence of a Component Stimulus subsequent to its Withdrawal.*

In the rhythmic reflex a contraction-phase (extension-phase of step) which is in course of execution does not cease immediately on withdrawal of the contralateral stimulus. On the contrary it continues its course for a brief time and ends in smooth transition and reversal into relaxation. This want of abruptness is strikingly different from the abruptness with which a reflex inhibition excited by an ordinary stimulus against tonus often commences. The excitatory phase thus continued after withdrawal of the contralateral stimulus is frequently less ample than its predecessors where the stimulus was not withdrawn (fig. 16, observation 2). A small final step to the series is thus produced (fig. 1, observation 1). If the contralateral stimulus be withdrawn during the course of the relaxation phase of the rhythmic reflex, *i.e.* during the flexion phase of the step, often no subsequent contraction-phase ensues (fig. 4, observations 3 and 4), even although the withdrawal occur late in the course of the relaxation phase (fig. 4, observation 3). But if the contralateral stimulus be relatively strong and the ipsilateral relatively weak, and the former be withdrawn just before the end of the relaxation phase, a small and somewhat delayed subsequent contraction phase may ensue (fig. 10, observation 3), and even be followed by one still smaller and still more delayed (fig. 1, observation 3).

Conversely with the withdrawal of the ipsilateral stimulus. If this cease during a relaxation phase that phase is completed more or less amply after the stimulus withdrawal (fig. 4, observation 1). If it be withdrawn during a contraction-phase no relaxation phase usually follows, but there may be just the commencement of one if the ipsilateral stimulus is withdrawn at end of a contraction phase (fig. 16, observation 1), and if the ipsilateral stimulus as compared with contralateral be not too weak.

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* 'Roy. Soc. Proc.,' 1911, B, vol. 84, p. 308.

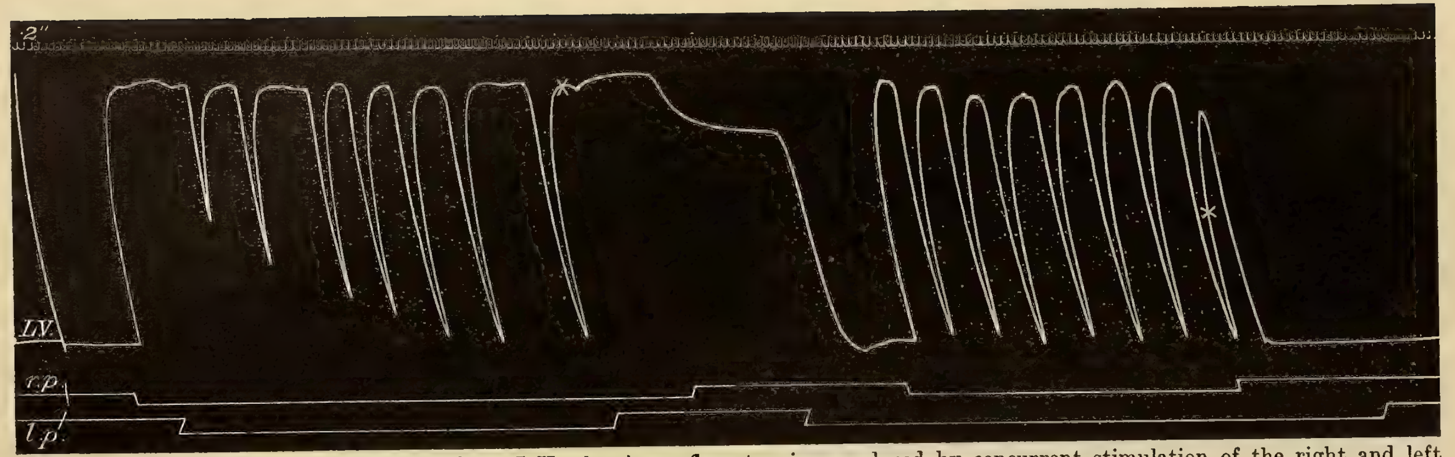

Fig. 16.—Isolated extensor muscle of left knee L.V., showing reflex stepping produced by concurrent stimulation of the right and left peroneal afferents, *r.p.* and *l.p.* The figure shows that one and the same stimulus combination does not work equally well on two successive occasions. In Observation 1 the figure illustrates the after-effect of the inhibitory stimulus subsequent to its withdrawal, its effect being the small notch occurring just after the cross. In Observation 2 the dwarfing of the contraction (extensor) phase after withdrawal of the excitatory stimulus just previously is shown. The moment of withdrawal of the stimulus is marked by a cross. Decerebrate cat. Time above in fifths of seconds.

IV. SUMMARY OF CONCLUSIONS.

It is shown that taking an afferent nerve which produces steady reflex excitation of the muscle, and another which produces steady reflex inhibition of the muscle, it is possible by stimulating both nerves concurrently to obtain regularly rhythmic contractions and relaxations of each member of a pair of symmetrical muscles, the phases being reciprocal in the two. To do this requires certain somewhat narrowly adjusted proportions of strength of the two paired stimuli. The stimuli are both of them continuous, in the sense that they are faradic and of a frequency (about 40 per second) much above and bearing no causal relation to the rhythmic reflex produced. In the rhythmic reflex the right and left muscles each contract and relax alternately and move reciprocally, the contracting phase of right muscle being synchronous with the relaxing phase of left, and conversely. This rhythmic reflex is shown clearly to be reflex stepping. In short, under the rivalry of the two opposed and so to say equipoised continuous stimulations th limbs exhibit reflex walking. With certain other paired intensities of stimulation of the two antagonistic afferent nerves, it can be arranged that only one muscle of the pair shall step—the left muscle if the right nerve stimulus be the stronger, and conversely. During this unilateral walking or running the other leg is kept steadily flexed by the reflex, *i.e.* the extensor muscles are kept steadily inhibited.

Herbage Studies. II.—*Variation in* Lotus corniculatus *and* Trifolium repens (*Cyanophoric Plants*).

By H. E. Armstrong, F.R.S., E. Frankland Armstrong, and
Edward Horton.

(Received January 1,—Read January 23, 1913.)

Lotus corniculatus.

In Part I of these studies, it is shown that *Lotus corniculatus* is a plant in which a glucoside containing cyanogen is frequently present together with the corresponding enzyme. During 1911 we were able to make observations practically over the whole of Europe, owing to the assistance we received from Dr. Eyre, which led us to the conclusion that the glucoside and enzyme were normal constituents of the plant in almost all districts, though occasionally, in close proximity to plants which were cyanophoric, others were met with in which little if any cyanide could be detected. In Scotland, in South Ayrshire, the plant was uniformly *acyanophoric,* except on the coast; nor could cyanide be detected in plants collected in Norway.

Lotus major, which is a sufficiently distinct variety to have been recognised by botanists as a separate species, was uniformly free from cyanide and also apparently from enzyme; but no regular distinction could be made between the various other forms which botanists look upon as merely varieties of the plant. *Lotus major*, it should be added, always affects damp situations and is a rank grower; it can be distinguished by the manner in which the large number of flowers in the umbel spread out from a common centre instead of at intervals from the flower stalk; the calyx teeth also tend to spread outwards, whereas in other forms they are almost uniformly strongly incurved. We have found the double form of *L. corniculatus* (var. *pleno*) to be strongly cyanophoric.

During 1912, we have again examined specimens of *L. corniculatus* from many localities in England and Wales and, as a rule, have found them to be strongly cyanophoric.* Out of about 30 specimens collected day by day by a cyclist during a fortnight's tour from London to Wales and back, in the earlier part of July, only two were found to be almost free from cyanide—one of these was obtained at Church Stretton, Somerset, the other from the foot of Cader Idris, Wales. Specimens sent to us by Mr. Pickering from Woolacombe, South Devon, and by Mr. Stapledon (*L. incanus*, Gray, *L. villosus*, B. and H.) from Westward Ho, North Devon, were strongly cyanophoric ; on the other hand, material sent to us from near the Lizard, Cornwall, gave no response to the test.

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* During 1912, in testing for cyanide, the Guignard picrate paper used was always prepared as required and we have substituted toluene for chloroform in order to avoid the possibility of any trace of acid being introduced. In the morning, before going into the field, strips of paper were impregnated with the alkaline picrate solution and the moist strips were at once placed in the tubes which were to be used in testing the plants. The tubes were then incubated in the pocket, in order to ascertain whether any hydrogen cyanide was retained in the cork. Recently prepared undried paper is usually more sensitive than paper which has been dried and then moistened just before it is to be used; the once dried paper rarely has the bright yellow appearance of freshly stained paper.

At the end of June, one of us found the plant growing very freely, in full bloom, on the retaining wall at the foot of the hill slope bordering the whole length of Rydal Water, Westmorland ; of seven specimens, presenting no difference in appearance, picked from this wall at fairly regular intervals, all but one were more or less strongly cyanophoric ; the exceptional specimen contained the faintest trace, if any, of cyanide.

During August, a very thorough study of the plant was undertaken by one of us, chiefly in the valley of the River Cree on the borders of Ayrshire, Kircudbrightshire and Wigtownshire, in the district where the previous year, at Whitsuntide, the specimens examined were all acyanophoric.

Again, in the places visited in the previous year, many specimens were found in which cyanide could not be detected ; here and there, however, along the river bank and in the adjoining fields, patches of the plant were met with now and then which were *faintly* cyanophoric.

On walking along the high road, across the moor, from Drumlamford, about five miles from Barrhill Station, to Newton Stuart, a distance of about 12 miles, a delicate stunted form of *Lotus corniculatus* was frequently found growing at the roadside among grass, *Lotus major* being plentiful in damp situations near Newton Stuart. Five specimens of the plant were secured in the course of 10 miles ; the first of these, obtained about three miles out, was strongly cyanophoric, the picrate paper being coloured brick red by the evening ; not a trace of hydrogen cyanide was observed in the case of the other four specimens. About two miles from Newton Stuart, where the River Cree comes into view, at the foot of a fairly steep hill, there was a profuse growth of *L. corniculatus* on the top of the retaining wall at the left hand side of the road ; several specimens were tested ; strange to say, none of them appeared to be cyanophoric.

Very faint indications of hydrogen cyanide were observed in some but not in all plants collected between Drumlamford and Barrhill Station. On walking from Pinwherry, the next station north of Barrhill, to the coast at Ballantrae, about eight miles, along the road passing through the valley of the Stincher, *Lotus major* was found to be abundant everywhere in the moist bank at the foot of the hill slope on the right. *L. corniculatus* was also met with here and there at the edge of the road ; specimens collected within the first, within the second and within the next two miles, all gave fair to strong indications of cyanide.

At Ballantrae, as in 1911, *L. corniculatus* was growing freely in very coarse sand and stones, on the upper level of the beach, in large clumps or compact tufts consisting of long straggling stems bearing small, delicate leaves but large seed-pods, the underground root growth being very strong ; cyanide was present in moderate amount in this plant. A short distance away, across the main road, at the foot of the hill, delicate plants with small seed pods were found growing in the grass beneath a wire fence bordering the rough roadway leading up hill. In 1911, nothing was detected in the plant in this situation but last year distinct indication of the presence of cyanide was obtained—perhaps only because the test applied was a more delicate one. In any case, the very different conditions prevailing on the shore and near at hand on the hillside had favoured the development of very different types of plant.

The contrast is equally striking when the plant growing on the beach at Ballantrae is compared with that found on the East coast near Dundee and St. Andrews on either side of the Tay estuary. *L. corniculatus* is abundant in these localities on the outer margin of the sand dunes, where it grows in fine blown sand. The growth is chiefly underground and is much less coarse in character than that at Ballantrae; usually, the leaves alone, which are very small and delicate, appear above ground. Cyanide was found to be present in this plant, though in very much smaller amount than in the coarse growing Ballantrae form.

Taking the observations made *in the field* last year and the previous year into account, it appeared to be little short of established that, in addition to the common widely distributed cyanophoric form of *L. corniculatus*, a botanically indistinguishable form exists, different from *L. major*, which, like this latter, is acyanophoric.

Considerable quantities of plant collected in the Cree valley were brought to London for the purpose of determining the enzymic activity. Among these were various samples in which cyanide had not been detected; when these were incubated at 37°, with a few drops of toluene, the presence of cyanide in the plant became obvious within 24 hours, though the amount detected was very small in all cases. On testing *L. major* in the same way, *no trace* of cyanide was detected.

It may be added that traces of cyanide have been found in plants grown during 1912 from seed gathered the previous year in Norway from plants in which cyanide could not then be detected.

Whilst therefore it appears that *L. major* is uniformly acyanophoric and that the common forms of *L. corniculatus* are more or less strongly cyanophoric, a form of this latter species undoubtedly exists in which the power of producing the cyanophoric glucoside is all but suppressed.

Enzymic Activity of L. corniculatus *from Various Localities.*

The determination of the enzymic activity of plants differing in habit and from various localities is obviously of importance in view of the probability that the cyanophoric glucoside and the enzyme are in close correlation.

It should be noted that the activity of *L. major* towards linamarin is so slight as to be negligible (see Part I) and it may almost be assumed that this species is not only acyanophoric but also free from the specific enzyme met with in *L. corniculatus*.

The enzymic activity of all the specimens of *L. corniculatus* examined during 1911 in which the cyanophoric glucoside was present was high. Of the four specimens from Norway, in which cyanide was not detected, however, one was moderately active, one but slightly active and two active.

The results obtained on examining specimens collected last year are as follows:—

Enzymic Activity of *Lotus corniculatus.*

Source of specimen.	Percentage hydrolysed.	
	Linamarin.	Salicin.
Moscow,* from American clover seed	84·2	—
,, ,, West Russian clover seed	80·5	—
Ballantrae (August 27, 1912)	57·0	20·0
River Cree, Ivy Pool (August 29, 1912)	74·0	31·0
Lane from Dalnaw Farm (September 1, 1912)	40·5	—
Face of bank below Farm, near bridge (September 1, 1912)	2·6 3·0	3·0
Edge of ditto (September 1, 1912)	71·7	28·0
Barry Links, Taymouth (September 8, 1912)	3·0 3·0	3·2
Pilmour Links, St. Andrews (October 9, 1912)	49·5 45·2	21·2
Lotus tenuis	—	50·4

* We are indebted to Prof. Williams for these samples; they are referred to in the table given in Part I.

It will be seen that there is a marked difference between the plants collected on opposite sides of the River Tay, near Dundee and at St. Andrews, the one having but little activity, the other being moderately active. So far as we were able to judge, the two plants were identical in external appearance.

Still more remarkable is the difference between Nos. 8 and 9. Both were strong plants showing faint traces of cyanide: the one specimen was taken from a large tuft overhanging the very edge of the bank; the other from a large patch at most a yard or two distant from the first, growing below it where the bank began to slope away to the river.

It appears, therefore, to be established that whilst the "normal form" of *L. corniculatus* generally met with in the southern parts of Britain contains both a cyanophoric glucoside and the correlated enzyme, in Scotland and also in Norway a form prevails which is "rich" in enzyme but contains mere traces of the glucoside; and that a third form exists in which the amount of enzyme is also very small. The second form appears to be widely distributed though less common than the first: but the third has been met with only rarely (once at home and perhaps in Norway). We hope that we shall be able to obtain further information, during the present year, which will enable us to throw more light on the nature of the relationship between the different varieties.

Inasmuch as the glucoside is sometimes all but absent in cases in which an apparently "normal" amount of enzyme is present, it appears probable that the presence of these two "factors," which undoubtedly are to be regarded as correlated, is not sufficient and that some other factor or factors are concerned in the production of the glucoside if not of the enzyme. As traces of cyanide are usually found, except in *L. major*, it is scarcely probable that the power to produce either cyanide or acetone is altogether lacking; it is more likely that the conditions of concentration may not be suitable and that the factor in question is one influencing concentration. In any case, taking into account the fact that plants possessed of very different characters have been found in close proximity, it is difficult to avoid the conclusion that the differences observed are less the consequence of the operation of special conditions of environment—though these may contribute—than of the presence or absence of definite factors.

Trifolium repens.

It was pointed out in the first part of these studies that our special object is the development of methods of appraising the value of the various constituents of the herbage of pasture lands. The study of *L. corniculatus* is of interest from this point of view, as the plant affords a striking case of variation under natural conditions and it is to be supposed that any light thrown on the causes of variation will be of assistance in dealing with the general problem of variation. But in other respects, the plant is not well suited to our purpose, as it ranks rather as a weed than as a fodder plant and is an altogether minor constituent of most pastures.

By far the most important leguminous plant in pastures is white clover, *Trifolium repens*. We have been the more attracted to this plant by the appearance of the account given by Hall and Russell of their exhaustive study of the remarkable conditions prevailing in Romney Marsh,* where pastures are found, side by side, one of which is good sheep-fattening land but unsuitable and unsafe for ewes with lambs, whilst the other has no fattening qualities and is used only in rearing lambs, which thrive on it. *Trifolium repens* is abundant in these pastures and is an important constituent of the herbage, particularly of the fattening fields and especially throughout the earlier part of the year.

Mirande has anticipated us in making known the presence of a cyanophoric glucoside in this plant.† In our experience, the wild plant, wherever tested, always contains cyanide; the amount is not large—from 0·0036 to 0·039 according to Mirande's determinations—and always below that present in the markedly cyanophoric forms of *L. corniculatus*.

It is noteworthy that seedsmen and agriculturists recognise two varieties of *Trifolium repens*—cultivated and wild white clover; and that of late years the latter has acquired some popularity as the more lasting variety. An interesting account of Wild White Clover, by Dr. A. Gilchrist, Professor of Agriculture in the Armstrong College, Newcastle-on-Tyne, was published in the 'Journal of the Board of Agriculture' in 1909 (vol. XVI, No. 91). As an illustration of the difference between this form and the commonly cultivated variety, Prof. Gilchrist cites an experiment made at Cockle Park Farm, Northumberland, in which two quarter-acre plots of the poorest type of boulder clay soil, laid down to grass in April 1906, each received together with other seeds 4 lbs. of cultivated or commercial white-clover seed per acre and one of them (Plot II), in addition, 4 lbs. per acre of the wild white-clover seed; the hay produced on the two plots was as follows:—

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* 'Journ. Agric. Sci.,' vol. iv, p. 339.

† 'Comptes Rendus,' 1912, vol. 155, p. 651.

	Weight of hay.	
	Plot I.	Plot II.
	cwt.	cwt.
1907	30½	35
1908	18¼	28¼
1909	15½	21¾
Average	21⅓	28½

To quote Prof. Gilchrist, "The aftermath has been grazed every year. White clover and practically all the clovers disappeared from Plot I after the first year but now some natural clover plants are spreading on this plot. Plot II has always had a thick and close sward of white clover and this continues to be so. It may be noted that on this cold clay soil meadow fescue seed has failed to produce plants. A striking result is that on Plot I the grasses have not been nearly so luxuriant as on Plot II. This was so even in the first year's hay crop and is undoubtedly due to the collection of nitrogen from the atmosphere by means of the nodules on the clover roots and to the stimulating effects of the nitrogen on the grasses."

We have not succeeded in finding cyanide in white clover raised from "cultivated" seed at any stage of growth. When the wild white seed is germinated, faint traces of cyanide can be detected even on the fourth or fifth day, as soon as the cotyledons begin to assume a green tinge, the response to the cyanide test being more distinct a day or so later when they are fully green though only just emerging from the seed husk.

Enzymic Activity of Trifolium repens.

The method adopted in estimating the enzymic activity was that described in Part XVII of our Studies on Enzyme Action involving the use of the dried leaf material.*

The following are the results obtained:—

Enzymic Activity of *Trifolium repens.*

	Linamarin.	Amygdalin.	Prunasin.	Salicin.
Rothamsted Park permanent grass plots—				
5^2	—	—	—	7·0
6	—	2·5	12·0	11·0
7	17·7	—	—	15·5
Romney Marsh—				
Westbroke	19·2	5·0	26·5	15·9
Blacklocks	19·2	—	—	14·7
Midley Paddock	—	—	—	18·6
Midley (poor)	—	—	—	13·9
Somerset—				
Non-scouring land (Nov. 1, 1912)	—	—	—	4·5
Scouring land (Nov. 9, 1912)	4·5	3·0	18·2	12·6
Non-scouring land (Nov. 9, 1912)	4·5	2·0	18·2	11·2
Armstrong College, County Demonstration Farm Kent	13·2	2·2 (average of 4 samples)	17·0	11·2 12·7 9·5 12·6
Kitchen End { Lawn	—	—	—	11·8
Kitchen End { Pasture	—	—	—	7·5
Woburn	—	—	—	13·1
Cultivated white (Sutton's seed)	1·0	—	2·5	11·7

The striking fact brought out in this Table is that, whereas with one exception all the specimens examined are moderately active towards salicin, the "cultivated" variety alone is practically without action on linamarin and prunasin. This observation is of special interest in connexion with the question raised in Part XVII of our Studies on Enzyme Action.

Apparently we are in face of differences similar to those which have been discovered in *L. corniculatus.*

Should this discovery be confirmed, it is obvious that the object we have in view will have been in part attained, as we shall be in possession of varieties of a plant which is acknowledged to be one of the most valuable constituents of the herbage of pasture land; it will be all important to ascertain whether the chemical peculiarities already recognised, presented by the two types of the plant, are accompanied by others and are in any way to be correlated, directly or indirectly, with their value as food materials. Hitherto, no distinction has been drawn between them in this respect, the wild form having been favoured of late years but only on account of its more perennial character.

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* 'Proc. Roy. Soc.,' B, vol. 85, p. 363.

We have to thank Messrs. Temperley and Co. of Hexham and Newcastle-on-Tyne for placing seed of the Wild White variety of Clover at our disposal and also Prof. Gilchrist, Mr. C. T. Gimingham, Dr. Russell, Prof. Somerville and Dr. J. Voelcker for the trouble they have taken in assisting us to procure specimens.

The Trypanosomes found in the Blood of Wild Animals Living in the Sleeping-Sickness Area, Nyasaland.

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; Majors DAVID HARVEY and A. E. HAMERTON, D.S.O., R.A.M.C.; Dr. J. B. DAVEY, Nyasaland Medical Staff;* and Lady BRUCE, R.R.C.

(Received January 13,—Read March 6, 1913.)

(Scientific Commission of the Royal Society, Nyasaland, 1912.)

INTRODUCTION.

The chief object of this Commission in coming to Nyasaland was to inquire into the relation of the African fauna to the maintenance and spread of trypanosome disease.

The Commission arrived at their camp on Kasu Hill on January 12, 1912. As this was the rainy season the low country was covered with dense vegetation and much of it under water. Nothing could therefore be done in the study of the fauna until about the beginning of June, when the dry season was well established.

The camp at Kasu is situated on one of the hills (lat. 13° 40′ S., long. 34° 12′ E.) which rise on the western edge of the flat country adjoining Lake Nyasa. This low-lying lake-coast plain looks quite flat when viewed from the camp, and extends from the lake shore some 20 miles inland. The camp lies about 10 miles from the edge of this low country, and, therefore, some 30 miles from the lake. This plain is covered with thorn scrub, except near the lake, where there are large grassy plains, or "dambos," dotted over with palm trees. The thorn-scrub is the home of the tsetse-fly and also of numerous wild animals.

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* Dr. Davey resigned his membership of the Commission in October, before the completion of the work here recorded.

When an animal is shot in this fly-country by a member of the Commission a small quantity of the blood is taken in a bottle containing citrate of soda solution for inoculation purposes, and a thick and thin film of the blood spread on glass slides for microscopical examination. The blood is then sent to a point on one of the main paths, where a motor-cyclist is waiting to carry it to the camp. When the blood arrives it is at once injected into a goat, a monkey, and a dog.

WILD ANIMALS LIVING IN A FLY-AREA AS HOSTS OR RESERVOIRS OF TRYPANOSOME DISEASE.

The following table represents the result of the examination of 180 specimens of wild game or other animals shot in this fly-area. In one column is given the number of hours between the taking of the blood and its inoculation, in others the result of the microscopical examination of the thick and thin film, and of the inoculation of the blood. A plus sign means that trypanosomes are present, a minus sign that they are absent, and a blank space that a microscopical specimen was not available for examination, or an animal for inoculation, as the case may be.

Table I.—List of Wild Animals living in a Fly-area whose Blood has been Examined for Trypanosomes.

Date.	Expt. No.	Animal.	Age of blood, in hours.	By microscopical examination.		By inoculation.		
				Thick.	Thin.	Goat.	Monkey.	Dog.
1912.								
Jan. 20 ...	32	Hartebeeste ...		—	—	—		—
,, 20 ...	33	,, ...			—	—		—
,, 20 ...	34	,, ...			—	—		—
,, 22 ...	40	,, ...			—	—		—
,, 22 ...	41	Eland			—	—		+
,, 22 ...	44	,, ...			—	+		—
,, 23 ...	62	Sable		—	—	—		—
,, 23 ...	63	,, ...				—		—
,, 26 ...	95	Eland		—	—	—		—
Feb. 6 ...	159	Warthog				—		—
,, 6 ...	160	Sable			—	—		—
,, 18 ...	246	Elephant			—	—		—
,, 23 ...	283	Eland			—			
,, 28 ...	288	Lion			—	—		—
May 19 ...	615	Oribi	3	—	—	—		—
,, 19 ...	616	Waterbuck ...	17		+	—		
,, 25 ...	583	Duiker			—	—	—	—
,, 25 ...	584	Warthog			—	—	—	—
,, 26 ...	589	,, ...			—			—
,, 26 ...	591	Reedbuck			+			—
,, 26 ...	593	Duiker			—			—

Table I—*continued.*

Date.	Expt. No.	Animal.	Age of blood, in hours.	By microscopical examination.		By inoculation.		
				Thick.	Thin.	Goat.	Monkey.	Dog.
1912.								
June 2 ...	611	Buffalo	9	–	–	–	–	–
,, 2 ...	612	,, ...	9	–	–	–	–	–
,, 2 ...	613	,, ...	9	+	–	–	–	–
,, 2 ...	614	,, ...	9	–	–	–	–	–
,, 15 ...	686	Warthog	9½	–	–	–	–	–
,, 15 ...	687	Oribi	9½	–	–	–	–	–
,, 16 ...	692	Reedbuck	8½	–	–	–	–	–
,, 16 ...	695	Sable	5	–	–	–	–	–
,, 23 ...	742	Warthog	9	–	–	–	–	–
,, 23 ...	742*a*	,, ...	9	–	–	–	–	–
,, 23 ...	743	Oribi	9	+	–	–	–	–
,, 23 ...	743*a*	,, ...	9	–	–	–	–	–
,, 25 ...	744	Duiker	7½	–	–	–	–	–
,, 27 ...	755	Bushbuck	11	–	–	–	–	–
,, 29 ...	768	Hartebeeste ...	6½	–	–	–	–	–
,, 29 ...	769	Reedbuck	5½	–	–		–	–
,, 29 ...	780	,, ...	2	–	–		–	–
,, 30 ...	783	,, ...	¼	+	+		+	+
July 1 ...	777	Hartebeeste ...	7			–	–	–
,, 1 ...	778	,, ...	7			–	–	–
,, 1 ...	779	,, ...	7			+	+	+
,, 2 ...	820	Oribi	7½		–			–
,, 3 ...	801	,, ...	7½	–	–	–	–	–
,, 3 ...	818	Warthog	11¾	–	–		–	
,, 4 ...	813	Hartebeeste ...	8	–	–	–	–	–
,, 4 ...	814	,, ...	8	–	–	–	–	–
,, 4 ...	815	,, ...	8	–	–	–	–	–
,, 4 ...	816	,, ...	8	–	–	–	–	–
,, 4 ...	817	,, ...	8	–	–	–	–	–
,, 5 ...	828	Reedbuck		+	–		–	–
,, 6 ...	825	Hartebeeste ...	8	–	–			
,, 6 ...	826	Warthog	7¾	+	+	–	–	–
,, 6 ...	827	Oribi	7¼	–	–	–	–	–
,, 8 ...	844	Hartebeeste ...	4¾	–	–	–	–	–
,, 8 ...	863	Oribi	1¼	–	–		+	–
,, 10 ...	859	Hartebeeste ...	9¾	–	–	–	–	–
,, 10 ...	860	Oribi	5¾	–	+	–	–	–
,, 10 ...	861	Warthog	6¾	–	–	–	–	–
,, 10 ...	862	Oribi	5¾	–	–	–	–	–
,, 11 ...	866	,, ...	7½	–	–	–	–	–
,, 11 ...	869	Warthog	8½	–	–	–	–	–
,, 11 ...	872	,, ...	7½	–	–	–	–	–
,, 11 ...	875	Hartebeeste ...	5¾	–	–	–	–	–
,, 20 ...	912	Reedbuck	7¼	–	–	+	–	–
,, 21 ...	918	Hartebeeste ...	8	–	–	–	–	–
,, 21 ...	919	Oribi	7	–	–	–	–	–
,, 21 ...	920	Warthog	5	–	–	–	–	–
,, 21 ...	920*a*	,, ...	5	–	–	–	–	–
,, 21 ...	920*b*	,, ...	5	–	–	–	–	–
,, 21 ...	921	Oribi	8	–	–	–	–	–
,, 21 ...	923	Warthog	10	–	–	–	–	–
,, 21 ...	925	Bushbuck	10	–	–	–	–	–
,, 22 ...	927	Hartebeeste ...	7¾	–	–	–	–	–
,, 22 ...	929	Warthog	5¾	–	–	–	–	–
,, 22 ...	931	,, ...	5¾	–	–	–	–	–

Table I—*continued.*

Date.	Expt. No.	Animal.	Age of blood, in hours.	By microscopical examination.		By inoculation.		
				Thick.	Thin.	Goat.	Monkey.	Dog.
1912.								
July 22 ...	933	Warthog	4½	+	+	−	−	−
,, 22 ...	935	,, ...	4½	−	−	−	−	−
,, 23 ...	955	Hyæna	8	−	−	+	−	−
,, 23 ...	956	,, ...	8	+	−	−	−	−
,, 23 ...	957	Hartebeeste ...	5	−	−	+	+	+
,, 23 ...	958	,, ...	3½	+	+	−	−	−
,, 25 ...	988	Reedbuck	6¾	+	+	+	−	−
,, 26 ...	993	Duiker	5½	−	−	−	−	−
,, 27 ...	1000	Hartebeeste ...	3½	+	−	+	+	+
,, 29 ..	1004	,, ...	5¼	−	−	−	−	−
,, 29 ...	1007	Duiker	4	+	−	−	−	−
,, 30 ...	1010	Hartebeeste ...	4	−	−	−	−	−
,, 30 ...	1013	Eland	4	+	+	+	+	+
Aug. 1 ...	1017	Oribi	8¼	−	−	−	−	−
,, 2 ...	1024	Sable	7¼	−	−	−	−	−
,, 2 ...	1027	Duiker	5¼	+	−	−	−	−
,, 4 ...	1044	Eland	6½	−	−	+	−	−
,, 5 ...	1045	Duiker	6¾	−	−	−	−	−
,, 5 ...	1048	Wild cat	3	−	−	−	−	−
,, 7 ...	1052	Warthog	5¾	−	−	−	−	−
,, 7 ...	1055	Wild cat	2¾	−	−	−	−	−
,, 11 ...	1058	Koodoo	3½	+	+	−	−	−
,, 11 ...	1061	Waterbuck ...	2¾	+	−	+	−	−
,, 11 ...	1064	Warthog	2¾	+	+	−	−	−
,, 12 ...	1067	Hyæna	4	−	−	−	−	−
,, 18 ...	1075	Waterbuck ...	5	−	−	−	−	−
,, 18 ...	1078	Bushbuck	3	+	+	+	−	−
,, 18 ...	1081	,, ...	3	+	−	−	−	−
,, 18 ...	1084	,, ...	3	+	+	+	+	+
,, 18 ...	1087	,, ...	3	+	+	+	−	−
,, 19 ...	1090	Oribi	7	−	−	−	−	−
,, 19 ...	1093	,, ...	7	−	−	−	−	−
,, 19 ...	1096	,, ...	7	−	−	+	−	−
,, 19 ...	1099	,, ...	7	−	−	−	−	−
,, 19 ...	1102	,, ...	7	−	−	−	−	−
,, 21 ...	1136	Warthog	5¾	−	−	−	−	−
,, 21 ...	1139	,, ...	5	+	+	+	−	−
,, 21 ...	1142	Hartebeeste ...	7½	−	−	+	−	+
,, 21 ...	1145	,, ...	6¾	−	−	−	−	−
,, 22 ...	1150	Reedbuck	8½	+	+	+	−	−
,, 22 ...	1153	,, ...	8½	+	−	+	−	−
,, 22 ...	1156	,, ...	7	+	−	+	−	−
,, 22 ...	1159	,, ...	7	−	−	−	−	−
,, 22 ...	1162	,, ...	6	+	−	+	−	−
,, 22 ...	1165	,, ...	6	−	−	−	−	−
,, 23 ...	1168	Warthog	7¾	−	−	−	−	−
,, 23 ...	1171	Wild cat	6¾	−	−	−	−	−
,, 23 ...	1174	Waterbuck ...	7	−	−	−	−	−
,, 24 ...	1177	,, ...	5	−	−	−	−	−
,, 24 ...	1180	,, ...	5	+	−	+	+	+
,, 24 ...	1183	Warthog	7½	−	−	−	−	−
,, 24 ...	1186	,, ...	6	+	−	+	−	−
,, 24 ...	1189	,, ...	6	+	−	−	−	−
,, 24 ...	1192	Oribi	7¼	−	−	−	−	−
,, 24 ...	1195	,, ...	7¼	−	−	−	−	−

Table I—*continued.*

Date.	Expt. No.	Animal.	Age of blood, in hours.	By microscopical examination.		By inoculation.		
				Thick.	Thin.	Goat.	Monkey.	Dog.
1912.								
Aug. 24 ...	1198	Porcupine	8½	−	−	−	−	−
„ 28 ...	1202	Eland	4	+	+	+		+
„ 28 ...	1203	Bushbuck	5	+	+	−	−	−
„ 28 ...	1205	Eland	4	−	−	−		−
„ 28 ...	1210	Waterbuck ...	4	+	−	+	+	+
„ 30 ...	1216	Bushbuck	8	+	−	−		−
Sept. 6 ...	1250	Koodoo	2	−	−	−	−	−
„ 6 ...	1254	Oribi	6¾	−	−	−	−	−
„ 7 ...	1261	Bushbuck	4¼	−	−	+	−	−
„ 7 ...	1264	Waterbuck ...	3¼	+	+	−	+	+
„ 7 ...	1268	Buffalo	6¼	−	−	−	−	−
„ 7 ...	1272	Hartebeeste ...	5¼	−	−	−	−	−
„ 7 ...	1276	Warthog	4	−	−	−	−	−
„ 7 ...	1281	Buffalo	9	−	−	−	−	−
„ 10 ...	1285	„	5	−	−	−	−	−
„ 10 ...	1289	Eland	8¼	−	−	−	−	−
„ 10 ...	1293	Warthog	12¾	−	−	−	−	−
„ 10 ...	1298	Buffalo	5	−	−	−	−	−
„ 11 ...	1304	„	3¼	−	−	+	−	−
„ 12 ...	1308	Warthog	6			+	−	−
„ 13 ...	1339	Waterbuck ...	6½	−	−	+	−	−
„ 13 ...	1343	Bushbuck	7	−	−	−	−	−
„ 13 ...	1347	Reedbuck	6	+	−	−	+	+
„ 13 ...	1351	„	5¾	−	−	−	−	−
„ 14 ...	1355	Hartebeeste ...	7½	−	−	−	−	−
„ 14 ...	1359	„ ...	7½	−	−	−	−	−
„ 14 ...	1363	Reedbuck	6	−	−	+	−	−
„ 16 ...	1368	Oribi	7¼	−	−	−	−	−
„ 16 ...	1372	„	7¼	−	−	−	−	−
„ 16 ...	1376	Elephant	20	−	−	−	−	−
„ 17 ...	1380	Koodoo	2½	−	−	+	−	−
„ 17 ...	1384	Warthog	6½	−	−	−	−	−
„ 18 ...	1388	Waterbuck ...	8	+	+	+	−	−
„ 18 ...	1392	Hartebeeste ...	5	−	−	−	−	−
„ 18 ...	1396	„ ...	5	−	−	−	−	−
„ 18 ...	1400	Oribi	4½	−	−	−	−	−
„ 20 ...	1406	Waterbuck ...	9	−	−	+	−	−
„ 20 ...	1410	„ ...	9	−	+	−	−	−
„ 20 ...	1414	Warthog	6¼	−	−	−	−	−
„ 20 ...	1418	Hartebeeste ...		−	−	−	−	−
„ 20 ...	1422	„ ...	9	−	−	−	−	−
„ 20 ...	1426	„ ...	7	−	−	−	−	−
„ 20 ...	1435	Reedbuck	9	−	−	−	+	+
„ 20 ...	1439	„	8½	−	−	−	−	−
„ 20 ...	1443	Oribi	7¾	−	−	−	−	−
„ 24 ...	1447	Waterbuck ...	14	−	−	−	−	−
„ 25 ...	1453	Hartebeeste ...	10½	+	−	−	−	+
Oct. 6 ...	1471	Eland	2	+	−	+	−	−
Nov. 10 ...	1577	Warthog	3½	−	−	−	−	−

Total 180. Infected with pathogenic trypanosomes 57 = 31·7 per cent.

In the above table an account is given of the examination of 180 wild animals shot in the fly-area adjoining the Commission's camp at Kasu.

This part of the country is situated in the proclaimed Sleeping-Sickness Area of Nyasaland, which extends from the Chirua river (lat. 13° 20′ S., long. 34° E.) in the north to the Lintipe river (lat. 13° 50′ S., long. 34° 30′ E.) in the south. It is bounded on the east by the Lake and on the west by the foot-hills. The area is about 50 miles from north to south and 25 from east to west. These figures are only approximate, as the available maps are far from correct. This is the only part of this country in which cases of the human trypanosome disease of Nyasaland, up to the present, have been found. It will be seen, then, that these animals were procured from the very heart of the Sleeping-Sickness Area.

Among the 180 animals, 57 were found to harbour pathogenic trypanosomes—31·7 per cent.

Table II gives the species of trypanosomes found in the 180 animals. Here a difficulty is encountered—the classification. The tendency in this branch of natural history, as in all others, is to multiply species.

In a previous paper* the trypanosome causing human trypanosome disease in Nyasaland was called *Trypanosoma rhodesiense*, on account of the presence of posterior-nuclear forms. This trypanosome agreed in all other respects with *Trypanosoma brucei*, the common trypanosome of wild animals in South Africa, and the cause of the tsetse-fly disease, or Nagana. In order to compare the two species of trypanosomes more closely, the Commission procured, by the kindness of Dr. A. Theiler, C.M.G., Pretoria, a strain of Nagana from the same spot in Zululand where it was first discovered in 1894. Much to the surprise of the Commission it was found that *T. brucei* has quite as large a proportion of posterior-nuclear forms as *T. rhodesiense*, and that the blunt-ended character is common to both species. The Commission is therefore driven to the conclusion that *T. rhodesiense* is neither more nor less than *T. brucei*, and that the human trypanosome disease of Nyasaland is Nagana.

To this it may be objected that Nagana has never been known to attack human beings. This has probably been due to faulty diagnosis, cases in man being returned as malaria.

The pathogenic trypanosomes then, found in the blood of wild animals in Nyasaland, up to the present, by the Commission are *T. brucei* (Plimmer and Bradford) vel *rhodesiense* (Stephens and Fantham), *T. pecorum*, *T. simiæ*, and *T. capræ* (Kleine). *T. ingens* is also found, but this trypanosome cannot, with our present knowledge, be considered a pathogenic species to man or domestic animals.

In Table II the plus sign means that the trypanosome named at the top of the column was present in the blood. The other plus signs signify that

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* 'Roy. Soc. Proc.,' 1912, B, vol. 85, p. 423.

Table II.—Species of Trypanosomes found in the Blood of Wild Animals living in the Sleeping-Sickness Area, Nyasaland.

Date.	Expt. No.	Animal.	*T. brucei* vel *rhodesiense.*	*T. pecorum.*	*T. simiæ.*	*T. capræ.*	*T. ingens.*	Thick film.	Thin film.	Inoculation.
1912.										
Jan. 22	41	Eland.........		+						+
,, 22	41	,, ...		+						+
May 19	616	Waterbuck				+			+	
,, 26	591	Reedbuck ...				+			+	
June 2	613	Buffalo ...		+				+		
,, 23	743	Oribi					+	+		
,, 30	783	Reedbuck ...	+			+		+	+	+
July 1	779	Hartebeeste	+							+
,, 5	828	Reedbuck ...				+	+	+		
,, 6	826	Warthog ...		+				+	+	
,, 8	863	Oribi	+							+
,, 10	860	,, ...				+			+	
,, 20	912	Reedbuck ...		+						+
,, 22	933	Warthog ...		+				+	+	
,, 23	955	Hyæna		+						+
,, 23	956	,, ...		+				+		
,, 23	957	Hartebeeste	+							+
,, 23	958	,,	+					+	+	
,, 25	988	Reedbuck ...				+		+	+	+
,, 27	1000	Hartebeeste	+					+		+
,, 29	1007	Duiker	+					+		
,, 30	1013	Eland.........		+		+		+	+	+
Aug. 2	1027	Duiker					+	+		
,, 4	1044	Eland.........		+						+
,, 11	1058	Koodoo		+				+	+	
,, 11	1061	Waterbuck		+		+		+		+
,, 11	1064	Warthog ...	+					+	+	
,, 18	1078	Bushbuck ...		+				+	+	+
,, 18	1081	,, ...		+				+		
,, 18	1084	,, ...		+				+	+	+
,, 18	1087	,, ...		+		+		+	+	+
,, 19	1096	Oribi		+						+
,, 21	1139	Warthog ...			+			+	+	+
,, 21	1142	Hartebeeste	+							+
,, 22	1150	Reedbuck ...				+		+	+	+
,, 22	1153	,, ...				+		+		+
,, 22	1156	,, ...				+		+		+
,, 22	1162	,, ...				+		+		+
,, 24	1180	Waterbuck	+			+		+		+
,, 24	1186	Warthog ...			+			+		+
,, 24	1189	,, ...		+				+		
,, 28	1202	Eland.........		+				+	+	+
,, 28	1203	Bushbuck ...		+				+	+	
,, 28	1210	Waterbuck	+			+		+		+
,, 30	1216	Bushbuck ...		+				+		
Sept. 7	1261	,, ...		+						+
,, 7	1264	Waterbuck	+					+	+	+
,, 11	1304	Buffalo		+						+
,, 12	1308	Warthog ...			+					+
,, 13	1339	Waterbuck				+				+
,, 13	1347	Reedbuck ...	+					+		+
,, 14	1363	,, ...				+				+
,, 17	1380	Koodoo ...		+						+
,, 18	1388	Waterbuck				+		+	+	+
,, 20	1406	,,				+				+
,, 20	1410	,,				+			+	
,, 23	1435	Reedbuck ...	+							+
,, 25	1453	Hartebeeste		+				+		+
Oct. 6	1471	Eland.........		+				+		+

the trypanosome was found in a thick or thin film or by inoculation of a quantity of blood from the wild animal into healthy experimental animals.

Table III.—Species of Trypanosomes found in the Blood of Wild Animals in the Sleeping-Sickness Area, Nyasaland, and the Number of Times each was found.

Number of animals.	*T. brucei* vel *rhodesiense.*	*T. pecorum.*	*T. simiæ.*	*T. capræ.*	*T. ingens.*
180	14	26	3	20	3

In every 100 wild animals living in the Sleeping-Sickness Area, Nyasaland, taken at random, the following numbers may be expected to be found infected with these species of trypanosomes.

Table IV.—Percentage of Animals infected by the different Species of Trypanosomes.

T. brucei vel *rhodesiense.*	*T. pecorum.*	*T. simiæ.*	*T. capræ.*	*T. ingens.*
7·8	14·4	1·7	11·1	1·7

Table V.—The Species of Animals dealt with, the Total Number examined, the Number found Infected, and the Species of Trypanosomes by which they were Infected.

Animal.	Total No. examined.	No. found infected.	*T. brucei* vel *rhodesiense.*	*T. pecorum.*	*T. simiæ.*	*T. capræ.*	*T. ingens.*
Eland	10	6		6		1	
Sable	5	0					
Waterbuck	13	9	3	1		8	
Koodoo	3	2		2			
Bushbuck ...	10	7		7		1	
Hartebeeste	35	6	5	1			
Reedbuck ...	19	12	3	1		9	1
Oribi	26	4	1	1		1	1
Duiker	7	2	1				1
Buffalo	9	2		2			
Lion	1	0					
Hyæna	3	2		2			
Elephant ...	2	0					
Warthog ...	33	7	1	3	3		
Wild cat ...	3	0					
Porcupine ...	1	0					
Total ...	180	59	14	26	3	20	3

The next table gives the percentages of the different trypanosomes occurring in the wild animals. The numbers are too small to be taken literally, but it is interesting to learn that in this fly-district the waterbuck, hartebeeste, reedbuck and duiker are dangerous neighbours to man; the eland, koodoo, bushbuck and buffalo to cattle, goats and sheep; and that the warthog is the only animal which harbours *T. simiæ*, the lightning destroyer of the domestic pig.

Table VI.—Percentages of Different Species of Trypanosomes harboured by Wild Animals in the Fly-area.

Animal.	No. examined.	*T. brucei* vel *rhodesiense.*	*T. pecorum.*	*T. simiæ.*	*T. capræ.*	*T. ingens.*
		per cent.	per cent.	per cent.	per cent.	per cent.
Eland	10		60		10	
Sable	5					
Waterbuck	13	23	8		61	
Koodoo	3		66			
Bushbuck	10		70		10	
Hartebeeste	35	14	3			
Reedbuck	19	16	5		47	5
Oribi	26	4	4		4	4
Duiker	7	14				14
Buffalo	9		22			
Lion	1					
Hyæna	3		66			
Elephant	2					
Warthog	33	3	9	9		
Wild cat	3					
Porcupine	1					

CONCLUSIONS.

1. 31·7 per cent. of the wild game in the fly-country below Kasu Hill harbour pathogenic trypanosomes.

2. The species of trypanosomes found are *T. brucei* vel *rhodesiense* 7·8 per cent., *T. pecorum* 14·4, *T. simiæ* 1·7, *T. capræ* 11·1, and *T. ingens* 1·7.

3. It is self-evident that these wild animals should not be allowed to live in "fly-country," where they constitute a standing danger to the native inhabitants and the domestic animals. It would be as reasonable to allow mad dogs to live and be protected by law in our English towns and villages. Not only should all game laws restricting their destruction in "fly-country" be removed, but active measures should be taken for their early and complete blotting out.

4. It must be strictly borne in mind that this only refers to wild animals living in fly-areas. No pathogenic trypanosomes have, up to the present, been found by the Commission in the blood of animals living in fly-free areas.

Trypanosome Diseases of Domestic Animals in Nyasaland.
II.—Trypanosoma capræ (*Kleine*).

By Surgeon-General Sir David Bruce, C.B., F.R.S., A.M.S.; Majors David Harvey and A. E. Hamerton, D.S.O., R.A.M.C.; Dr. J. B. Davey, Nyasaland Medical Staff;* and Lady Bruce, R.R.C.

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(Scientific Commission of the Royal Society, Nyasaland, 1912.)

[Plate 5.]

Introduction.

This species belongs to the *vivax* group, which consists of three species:—*Trypanosoma uniforme*, *T. vivax*, and *T. capræ.* They are all characterised by their extreme motility; clear cell contents; large, round, terminal micronucleus; and lastly, by the fact that the *vivax* group only infects cattle, goats, and sheep, and is harmless to the smaller laboratory animals. All three develop in the proboscis of the tsetse flies and not in the alimentary tract, as do other pathogenic trypanosomes.

T. vivax is stated to be pathogenic to horses, mules, and donkeys, but there has been no opportunity of testing these animals at Kasu with *T. capræ.*

It is curious that *T. uniforme* and *T. vivax* have not been met with by the Commission in Nyasaland. This may be due to the absence of *Glossina palpalis,* which is their carrier, while *T. capræ* is carried by *G. morsitans.*

Morphology of T. capræ.

A. *Living, Unstained.*

The description given of *T. vivax* can be equally applied to this species. It is just as active in its movements and dashes across the field of the microscope with the same impetuosity.

B. *Fixed and Stained.*

The blood films were fixed, stained, and measured as previously described in the 'Proceedings.'†

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* Dr. Davey resigned his membership of the Commission in October, before the completion of the work here recorded.

† 'Roy. Soc. Proc.,' 1909, B, vol. 81, pp. 16 and 17.

Length.—The following table gives the length of this trypanosome as found in the waterbuck, ox, goat, and sheep—500 trypanosomes in all.

Table I.—Measurements of the Length of *Trypanosoma capræ,* Nyasaland.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912.							
Sept. 18...	1388	Waterbuck ...	Osmic acid	Giemsa	26·8	29·0	25·0
„ 4...	349	Ox	„	„	25·9	32·0	22·0
„ 9...	350	„	„	„	25·5	30·0	18·0
March 4...	175	Goat	„	„	23·3	28·0	20·0
„ 11...	263	„	„	„	26·1	29·0	21·0
„ 14...	263	„	„	„	27·5	30·0	24·0
„ 18...	200	„	„	„	26·2	30·0	22·0
„ 20...	263	„	„	„	26·9	30·0	22·0
„ 21...	247	„	„	„	23·7	26·0	20·0
„ 21...	263	„	„	„	24·4	29·0	21·0
April 4...	339	„	„	„	25·1	28·0	23·0
„ 22...	272	„	„	„	23·4	27·0	21·0
„ 22...	339	„	„	„	24·6	27·0	21·0
„ 29...	339	„	„	„	27·6	31·0	25·0
May 13...	339	„	„	„	25·5	29·0	23·0
„ 16...	339	„	„	„	24·4	27·0	23·0
April 8...	348	Sheep	„	„	26·6	30·0	24·0
„ 11...	346	„	„	„	24·6	28·0	21·0
„ 11...	348	„	„	„	26·7	29·0	24·0
„ 25...	348	„	„	„	23·9	27·0	22·0
May 9...	348	„	„	„	26·2	29·0	24·0
„ 13...	348	„	„	„	24·4	27·0	22·0
„ 23...	347	„	„	„	24·7	27·0	22·0
June 13...	548	„	„	„	25·6	29·0	23·0
July 25...	907	„	„	„	27·5	32·0	24·0
					25·5	32·0	18·0

The average length of *T. capræ,* Nyasaland, in different species of animals, taken from Table I, is as follows:—

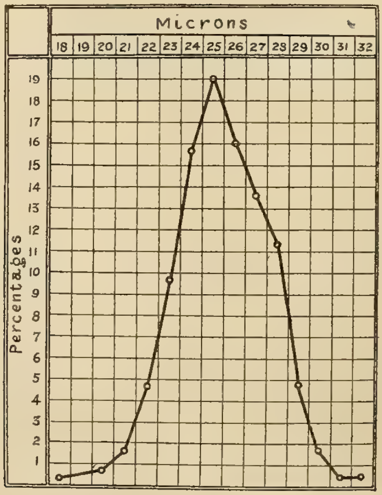
Table II.

Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Waterbuck	20	26·8	29·0	25·0
Ox	40	25·7	32·0	28·0
Goat.................	260	25·3	31·0	20·0
Sheep	180	25·6	32·0	21·0

Table III.—Distribution in respect to Length of 500 Individuals of *T. capræ*, Nyasaland.

Animal.	In microns.															Average length.
	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	
Waterbuck	—	—	—	—	—	—	—	1	9	4	4	2	—	—	—	26·8
Ox	—	—	—	—	1	2	3	2	3	6	2	—	—	—	1	25·9
,, ...	1	—	—	—	1	—	4	3	4	2	4	—	1	—	—	25·5
Goat	—	—	2	—	6	4	1	5	1	—	1	—	—	—	—	23·3
,, ...	—	—	—	1	—	3	1	2	2	5	4	2	—	—	—	26·1
,, ...	—	—	—	—	—	—	1	—	3	6	6	2	2	—	—	27·5
,, ...	—	—	—	—	1	1	3	4	2	1	5	2	1	—	—	26·2
,, ...	—	—	—	—	1	—	—	4	2	5	3	4	1	—	—	26·9
,, ...	—	—	1	2	—	6	4	3	4	—	—	—	—	—	—	23·7
,, ...	—	—	—	1	—	4	6	6	1	1	—	1	—	—	—	24·4
,, ...	—	—	—	—	—	4	5	4	2	2	3	—	—	—	—	25·1
,, ...	—	—	—	2	3	6	4	4	—	1	—	—	—	—	—	23·4
,, ...	—	—	—	1	—	2	7	4	5	1	—	—	—	—	—	24·6
,, ...	—	—	—	—	—	—	—	2	4	3	4	5	1	1	—	27·6
,, ...	—	—	—	—	—	2	2	7	5	2	1	1	—	—	—	25·5
,, ...	—	—	—	—	—	3	9	5	2	1	—	—	—	—	—	24·4
Sheep	—	—	—	—	—	—	3	4	1	6	3	1	2	—	—	26·6
,, ...	—	—	—	1	2	2	4	4	5	1	1	—	—	—	—	24·6
,, ...	—	—	—	—	—	—	1	2	7	3	6	1	—	—	—	26·7
,, ...	—	—	—	—	4	4	5	5	1	1	—	—	—	—	—	23·9
,, ...	—	—	—	—	—	—	1	4	8	4	2	1	—	—	—	26·2
,, ...	—	—	—	—	2	1	6	9	1	1	—	—	—	—	—	24·4
,, ...	—	—	—	—	2	2	3	8	3	2	—	—	—	—	—	24·7
,, ...	—	—	—	—	—	3	5	—	4	5	2	1	—	—	—	25·6
,, ...	—	—	—	—	—	—	1	3	1	5	6	1	1	1	1	27·5
Total	1	—	3	8	23	49	79	95	80	68	57	24	9	2	2	
Percentages ...	0·2	—	0·6	1·6	4·6	9·8	15·8	19·0	16·0	13·6	11·4	4·8	1·8	0·4	0·4	

CHART giving Curve representing the Distribution by Percentages in respect to Length of 500 Individuals of *Trypanosoma capræ*, Nyasaland.



This curve is made up of measurements from 20 specimens of trypanosomes taken from the waterbuck, 40 from the ox, 260 from the goat, and 180 from the sheep.

From it will be seen that *T. capræ* is a monomorphic species, varying from 18 to 32 microns in length, the greatest number of individuals (19 per cent.) being 25 microns long.

Breadth.—Measured across the broadest part *T. capræ* averages 3 microns in breadth (maximum 4·25, minimum 1·75).

Shape.—*T. capræ* differs from *T. vivax* in that it is heavier built and altogether has a larger and clumsier appearance. The posterior half is swollen, and its end is bluntly angular or rounded. The anterior extremity is narrower and pointed (Plate 5).

Contents of Cell.—Clear, with a delicate alveolar structure, and free from vacuoles or granules.

Nucleus.—Oval, compact, lying about the middle of the body.

Micronucleus.—Large and round, situated, as a rule, close to the posterior extremity, but sometimes removed to a short distance.

Undulating membrane.—Much more developed than in *T. vivax*, and thrown into bolder folds and undulations.

Flagellum.—There is a well-marked free flagellum which averages 6·5 microns in length (maximum 9·5, minimum 4). No specimens have been seen without a free flagellum as stated by Kleine.

Disease set up in Cattle by T. capræ.—Only two oxen were inoculated from goats suffering from this disease. These animals showed the trypanosomes in their blood in small numbers for two months after inoculation. The trypanosomes then disappeared and have never reappeared. The two oxen at the present time are in good health and have evidently recovered. This strain of *T. capræ* cannot, therefore, be considered of much pathological importance as far as oxen are concerned, but more cases are wanted. Kleine states that cattle are immune.

Disease set up in Goats and Sheep by T. capræ.—In goats and sheep, on the other hand, *T. capræ* runs a fairly fatal course. In the list of animal experiments 36 goats and 4 sheep are given. Of the 36 goats, 15 had been infected by wild *G. morsitans* and died, on an average of, from 53 to 59 days. As the flies were fed on a goat, a monkey, and a dog, and, as a rule, three times on each animal, to ensure that all the flies fed, it is not possible to tell the exact day of infection.

Four others were inoculated with blood from infected goats or antelope, and these died, on an average, in 57 days. The remaining 17 goats are still alive after intervals of from 61 to 262 days. Of the four sheep, one died in

36 days, another lived 89 days, the third 221 days, while the fourth is still alive after 245 days.

Table IV.—Animals Susceptible to *T. capræ*.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration, in days.	Remarks.
			Cattle.		
1912.					
Mar. 20...	349	From Goat 263	20	—	Alive and well after 245 days.
„ 20...	350	„ 263	10	—	These animals appear to have recovered.
			Goat.		
Feb. 6...	273	Wild flies	0–12	41–53	Died of *T. capræ*.
„ 14...	200	„	20–26	128–134	„ „
„ 22...	281	„	2–17	51–66	„ „
Mar. 3...	272	„	21–29	—	Alive after 262 days.
„ 11...	263	Natural infection	?	?	Died March 22.
„ 14...	334	Wild flies	7 ?	—	Alive after 251 days.
„ 16...	335	From Goat 263	5	—	„ 249 „
„ 16...	339	„ 263	9	63	Died of *T. capræ*.
„ 16...	340	„ 263	16	—	Alive after 249 days.
„ 20...	346	„ 263	5	—	„ 245 „
„ 20...	347	„ 263	12	—	„ 245 „
„ 20...	348	„ 263	5	89	Died of *T. capræ*.
Apr. 1...	398	Wild flies	10–21	101–112	„ „
„ 11...	410	„	6–11	46–51	„ „
„ 12...	412	„	0–13	0–20	„ „
„ 16...	414	„	7–13	33–39	„ „
„ 19...	422	„	4–10	—	Alive after 215 days.
„ 24...	415	„	13–19	46–52	Died of *T. capræ*.
„ 24...	420	„	13–19	43–49	„ „
May 2...	433	„	10	63	„ „
„ 4...	435	„	13–19	55–61	„ „
„ 9...	266	„	6–11	69–74	„ „
„ 9...	269	„	10–18	18–26	„ „
„ 18...	553	„	6–16	—	Alive after 186 days.
„ 23...	565	„	3–10	52–59	Died of *T. capræ*.
June 5...	622	„	15	34	„ „
July 25...	979	From Reedbuck 988	11	—	Alive after 118 days.
Aug. 18...	1039	From Bushbuck 1087	8	33	Died of *T. capræ*.
„ 22...	1111	From Reedbuck 1153	11	43	„ „
„ 22...	1114	„ 1156	11	—	Alive after 90 days.
„ 22...	1118	„ 1150	11	—	„ 90 „
„ 22...	1120	„ 1162	7	—	„ 90 „
Sept. 13...	1342	From Waterbuck 1339	20	—	„ 68 „
„ 14...	1366	From Reedbuck 1363	9	—	„ 67 „
„ 18...	1391	From Waterbuck 1388	8	—	„ 63 „
„ 20...	1409	„ 1406	17	—	„ 61 „
			Sheep.		
July 17...	907	From Goat 653	5	36	Died of *T. capræ*.
Mar. 20...	346	„ 263	5	—	Alive after 245 days.
„ 20...	347	„ 263	12	221	Died of *T. capræ*.
„ 20...	348	„ 263	5	89	„ „

Table IV—*continued.*

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration, in days.	Remarks.
		Monkey.			
1912.					
Feb. 2...	49	Wild flies	—	—	Only showed *T. simiæ.*
Mar. 9...	326	From Goat 175	—	—	Never showed trypanosomes.
Apr. 15...	405	Wild flies	—	—	Only showed *T. simiæ.*
” 23...	465	”	—	—	” ”
” 23...	467	”	—	—	Only showed *T. pecorum.*
July 25...	989	From Reedbuck 988 ...	—	—	Never showed trypanosomes.
Aug. 22...	1154	” 1153...	—	—	” ”
” 22...	1157	” 1156...	—	—	” ”
Sept. 13...	1340	From Waterbuck 1339	—	—	” ”
” 14...	1364	From Reedbuck 1363...	—	—	” ”
” 18...	1389	From Waterbuck 1388	—	—	” ”
” 20...	1407	” 1406	—	—	” ”
		Dog.			
Mar. 9...	319	From Goat 175	—	—	Never showed trypanosomes.
” 9...	320	” 175	—	—	Only showed *T. brucei.*
” 9...	321	” 125	—	—	Only showed *T. pecorum.*
” 9...	322	” 125	—	—	” ”
” 9...	344	” 263	—	—	Never showed trypanosomes.
” 9...	345	” 263	—	—	” ”
July 25...	990	From Reedbuck 988...	—	—	” ”
Aug. 22...	1155	” 1153...	—	—	” ”
” 22...	1158	” 1156...	—	—	” ”
Sept. 13...	1341	From Waterbuck 1339	—	—	” ”
” 14...	1365	From Reedbuck 1363...	—	—	” ”
” 18...	1390	From Waterbuck 1388	—	—	” ”
		Guinea-pig.			
Mar. 20...	351	From Goat 263	—	—	Never showed trypanosomes.
” 20...	352	” 263	—	—	” ”
		Rat.			
Mar. 20...	351	From Goat 263	—	—	Never showed trypanosomes.
” 20...	352	” 263	—	—	” ”

THE CARRIER OF T. CAPRÆ.

The carrier of *T. capræ* in Nyasaland is *G. morsitans.* These tsetse flies in the neighbourhood of Kasu are heavily infected with this trypanosome.

In the experiments made to ascertain with what trypanosomes the wild flies are naturally infected, *T. capræ* was found in 61 per cent.

The development of this trypanosome in *G. morsitans* will be dealt with in a future paper; suffice it to say here that it is restricted to the proboscis and runs a course of from 16 to 20 days.

The Host or Reservoir of T. CAPRÆ.

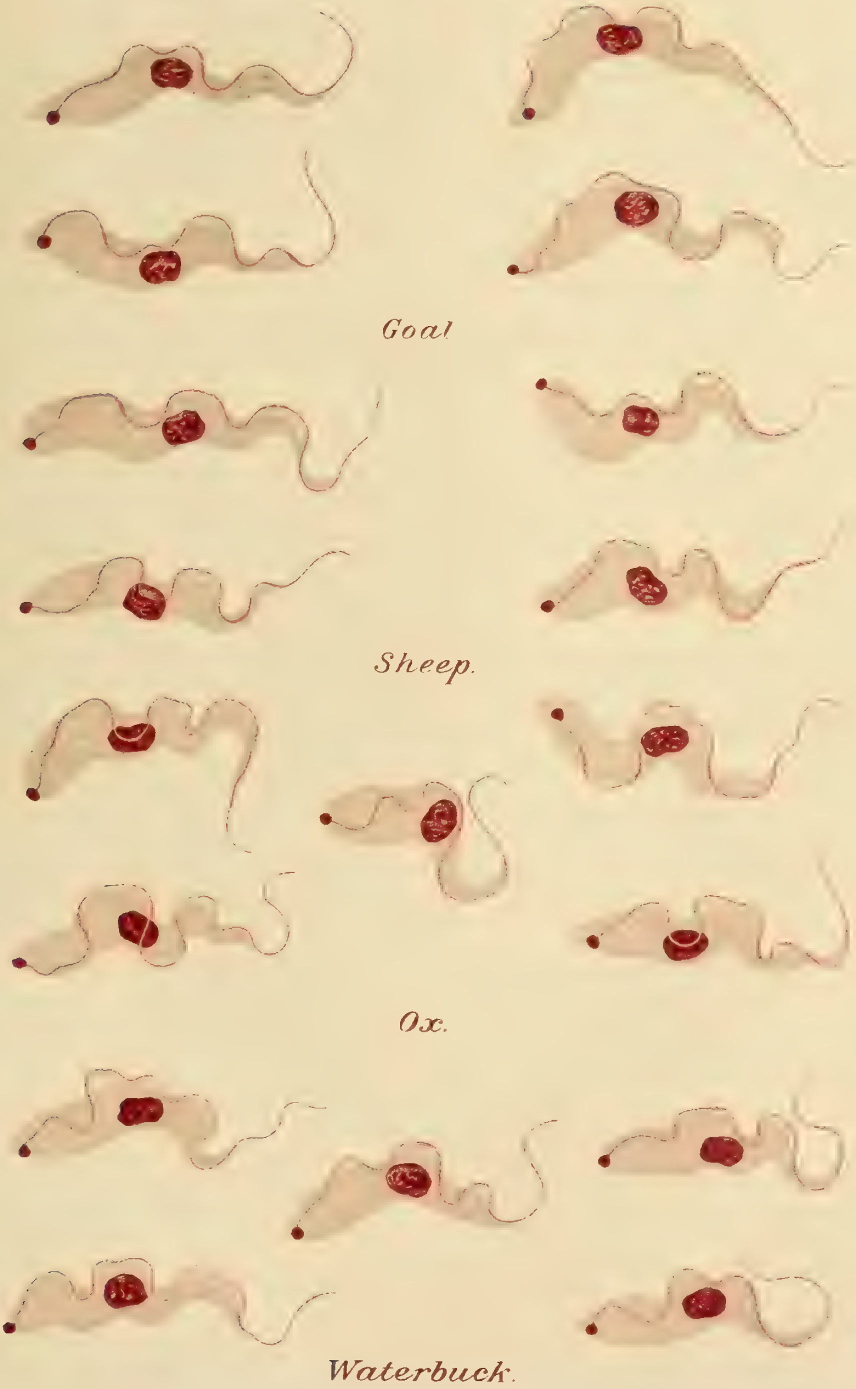
Up to the present 180 specimens of wild game living in the Nyasaland Sleeping-Sickness Area have been examined. Of these 19, or 10·5 per cent., harboured *T. capræ*. The animals were reedbuck, waterbuck, eland, and bushbuck.

Conclusions.

1. *T. capræ* belongs to the same group as *T. vivax* and *T. uniforme*, and affects the same animals—cattle, goats, and sheep. Monkeys, dogs, and the smaller laboratory animals are immune.
2. The carrier is *G. morsitans*.
3. The reservoir of the virus is the wild game living in the "fly-country."

DESCRIPTION OF PLATE.

Trypanosoma capræ (Kleine).—Large, heavily built body; posterior extremity swollen, bluntly angular, or rounded; anterior extremity pointed; nucleus oval, compact; micronucleus large, round, situated, as a rule, close to posterior extremity; undulating membrane marked, thrown into bold folds; flagellum well marked, free, average 6·5 microns in length. × 2000.


Goat
Sheep.
Ox.
Waterbuck.

Trypanosoma capræ (Kleine).

M. E. Bruce, del.

Morphology of Various Strains of the Trypanosome causing Disease in Man in Nyasaland. I.—The Human Strain.

By Surgeon-General Sir David Bruce, C.B., F.R.S., A.M.S.; Majors David Harvey and A. E. Hamerton, D.S.O., R.A.M.C.; and Lady Bruce, R.R.C.

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(Scientific Commission of the Royal Society, Nyasaland, 1912.)

Introduction.

In order to gain a general idea of this important species of trypanosome, it will be necessary to study as many individual strains as possible. It may be thought unnecessary to describe each strain so much in detail, but without this it will be impossible to get any order out of the chaos which rules at present in the classification of the African species of trypanosomes pathogenic to man and the domestic animals.

Up to the present the Commission have only had an opportunity of working with five human strains. Four of these are from natives infected in the Sleeping-Sickness Area, Nyasaland, the fifth from an European who contracted the disease in Portuguese East Africa. It is intended, in later papers, to describe five strains from wild game and the same number from the tsetse fly, *Glossina morsitans.*

The human strains are named: I, Mkanyanga; II, E——; III, Chituluka; IV, Chipochola; and V, Chibibi.

I. *Morphology of Strain I, Mkanyanga.*

This has already been dealt with in a previous paper.*

II. *Morphology of Strain II, E——.*

The following table gives the average length of this trypanosome as found in goats, sheep, monkeys, dogs and rats, 1500 trypanosomes in all, and also the length of the longest and shortest:—

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* 'Roy. Soc. Proc.,' 1912, B, vol. 85, p. 423.

Table I.—Measurements of the Length of the Trypanosome of Strain II, E——.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	22·2	36·0	15·0

The average length of the trypanosome of Strain II, in different species of animals, is as follows:—

Table II.

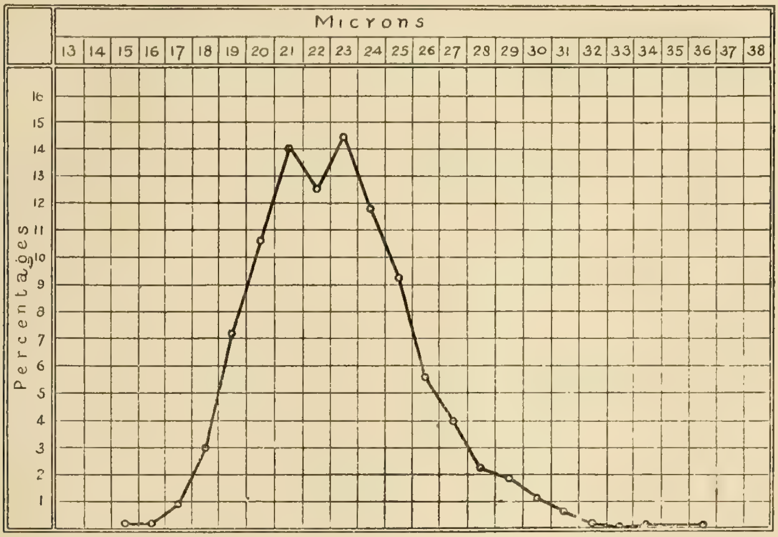
Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Goat	60	20·7	34·0	15·0
Sheep	20	21·3	28·0	18·0
Monkey	160	22·9	36·0	17·0
Dog	260	21·8	31·0	17·0
Rat	1000	23·1	32·0	17·0

Table III.—Distribution in respect to Length of 1500 Individuals of the Trypanosome of Strain II, E——.

	In microns.										
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.
Total	2	2	12	55	108	159	210	188	215	177	138
Percentage...	0·2	0·2	0·9	3·0	7·2	10·6	14·0	12·6	14·4	11·8	9·2

	In microns.										
	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.
Total	83	60	34	26	18	8	3	—	1	—	1
Percentage...	5·6	4·0	2·3	1·8	1·2	0·6	0·2	—	0·1	—	0·1

Chart 1.—Curve representing the Distribution, by Percentages, in respect to Length, of 1500 Individuals of the Trypanosome of Strain II, E——.



This curve is made up of measurements from 60 specimens of trypanosomes taken from the goat, 20 from the sheep, 160 from the monkey, 260 from the dog, and 1000 from the rat.

In a previous paper it was suggested that 1000 trypanosomes taken at random would be a suitable number to plot a curve from, for purposes of comparison. This is done in Chart 2.

The taking away of 500 rat trypanosomes has changed, to a great extent, the character of the curve. There is no resemblance between this curve and that given on Chart 1 of Strain I, Mkanyanga. If the two strains, I and II, belong to the same species, then little help can be expected from this system of measurement in classifying trypanosomes.

It has been suggested by Dr. J. W. W. Stephens that the measurements should be made from one animal, and he proposed the tame rat as a suitable species. There seems much to be said in favour of this. Practically, his proposal is that a series of slides should be made with blood taken on 10 consecutive days from a single rat, and that 100 trypanosomes should be drawn each day. But it is no light task to draw 1000 trypanosomes at a magnification of 2000, and afterwards to measure them. We have therefore made a compromise and measure 60 trypanosomes on nine consecutive days, beginning from the day the parasites first appear in the blood. In order to deal with a round number (500) only 20 are measured on the ninth day.

Chart 2.—Curve representing the Distribution, by Percentages, in respect to Length, of 1000 Individuals of the Trypanosome of Strain II, E——.

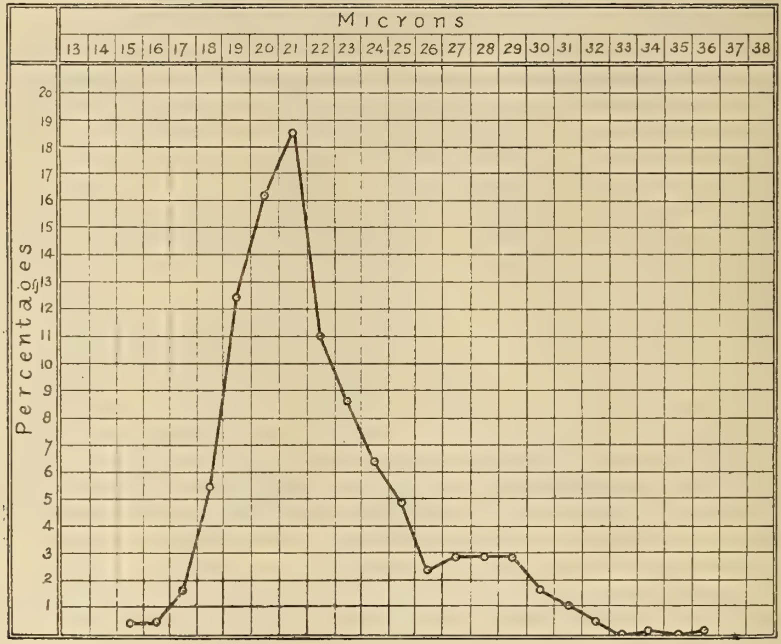
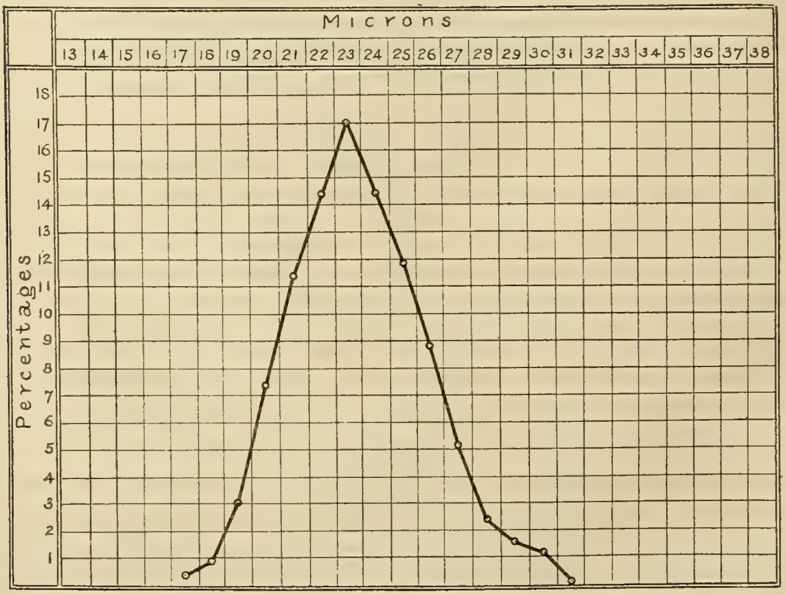


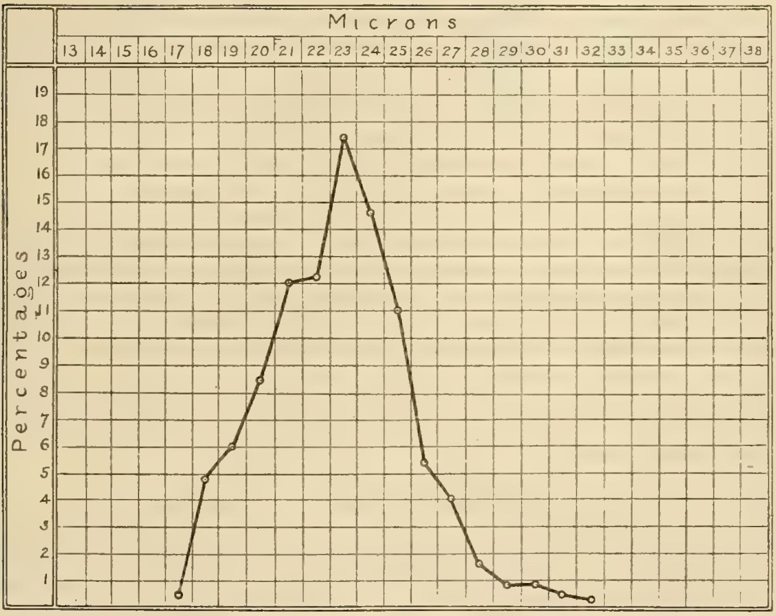
Chart 3.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain II, E——, taken on nine consecutive days from Rat 728.



This makes a symmetrical curve, which ascends and descends by fairly regular steps, but with little likeness to Charts 1 and 2.

In an organism low in the scale of nature, such as this, subject to great variation in form, it might be thought that it would not be likely to behave in any two rats in the same way. The following chart shows that this is not so, but that, on the contrary, the same strain of trypanosome planted in two different animals of the same species grows in a remarkably similar way.

Chart 4.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain II, E——, taken on nine consecutive days from Rat 726.



It is remarkable how much alike these last two curves are. If curves made in this way from different strains of one species of trypanosome showed the same degree of similarity, this method would certainly be useful for purposes of classification. But, as we have seen, the curve of Strain II has no resemblance to that of Strain I, and it will be found that each human strain of this species of trypanosome differs, more or less, when subjected to this method of measurement.

As the occurrence of posterior-nuclear forms has been made the distinguishing character between *Trypanosoma brucei*, *gambiense*, and *rhodesiense*, it will be of interest to note the percentage of these forms in the various strains. The method used is to count the number of posterior nuclears in 1000 short and stumpy forms in 10 specimens of a single rat's blood taken, as near as possible, on 10 consecutive days.

Table IV.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain II, E——.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
June 25	728	Rat	10
„ 26	728	„	17
„ 27	728	„	3
„ 29	728	„	9
July 1	728	„	5
„ 2	728	„	5
„ 3	728	„	9
„ 4	728	„	3
„ 5	728	„	18
„ 6	728	„	14
		Average	9·3

In regard to breadth, shape, contents of cell, nucleus, micronucleus, undulating membrane and flagellum, it is not proposed to describe these characters separately for each strain, as was done in Strain I. Suffice it to say that no difference can be made out in regard to these particulars on comparing the five strains. The same posterior-nuclear and blunt-ended forms are present in all.

III. *Morphology of Strain III, Chituluka.*

The following table gives the average length of this trypanosome as found in the goat, monkey, dog and rat, 1500 trypanosomes in all, and also the length of the longest and shortest :—

Table V.—Measurements of the Length of the Trypanosome of Strain III, Chituluka.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	26·1	38·0	15·0

The average length of the trypanosome of Strain III, in different species of animals, is as follows :—

Table VI.

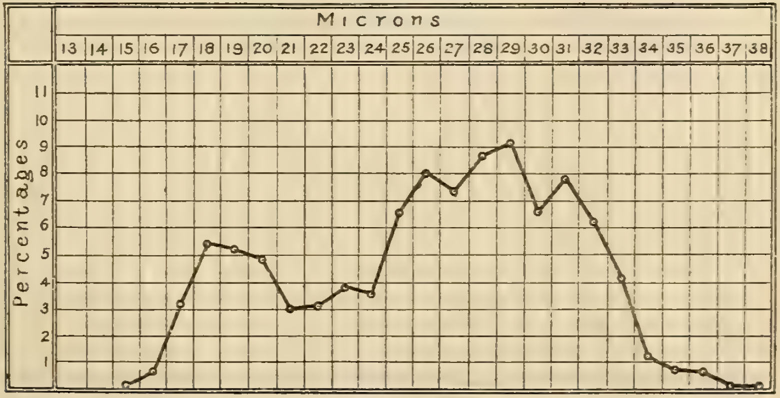
Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Goat	80	26·9	32·0	16·0
Monkey	160	27·7	36·0	16·0
Dog	260	24·1	35·0	16·0
Rat	1000	26·4	38·0	15·0

Table VII.—Distribution in respect to Length of 1500 Individuals of the Trypanosome of Strain III, Chituluka.

	In microns.											
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.
Total	1	8	48	81	78	71	44	46	56	53	98	120
Percentages	0·1	0·6	3·2	5·4	5·2	4·8	3·0	3·1	3·8	3·6	6·6	8·0

	In microns.											
	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.	37.	38.
Total	111	128	138	99	117	91	63	27	11	9	1	1
Percentages	7·4	8·6	9·2	6·6	7·8	6·2	4·2	1·1	0·7	0·6	0·1	0·1

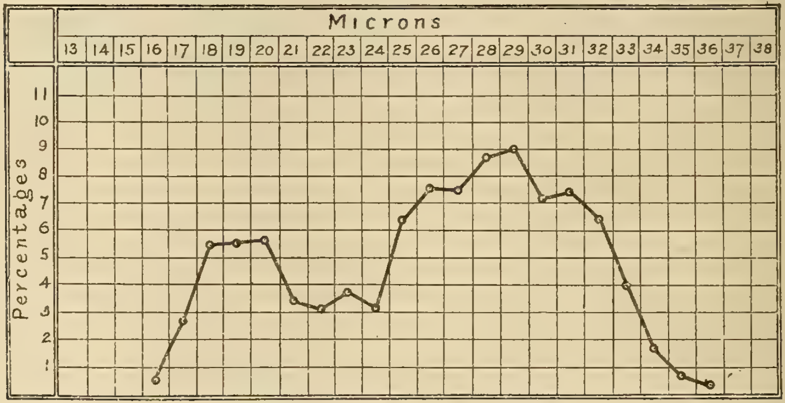
CHART 5.—Curve representing the Distribution, by Percentages, in respect to Length, of 1500 Individuals of the Trypanosome of Strain III, Chituluka.



This curve is made up of measurements from 80 specimens of trypanosomes taken from the goat, 160 from the monkey, 260 from the dog, and 1000 from the rat. It resembles that of Strain I, and differs absolutely from Strain II.

As in the case of Strain II, E——, a curve is also given of 1000 individuals of this strain.

Chart 6.—Curve representing the Distribution, by Percentages, in respect to Length, of 1000 Individuals of the Trypanosome of Strain III, Chituluka.



This curve, made up of 1000 individuals, is very similar to the previous one of 1500. It is made up of 80 specimens of trypanosomes taken from the goat, 160 from the monkey, 260 from the dog, and 500 from the rat.

The two following curves represent measurements of 500 trypanosomes taken from each of two rats.

Chart 7.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain III, Chituluka, taken on nine consecutive days from Rat 952.

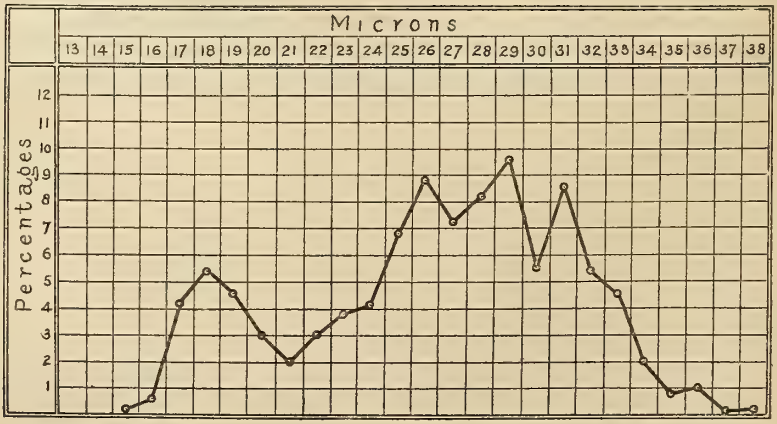
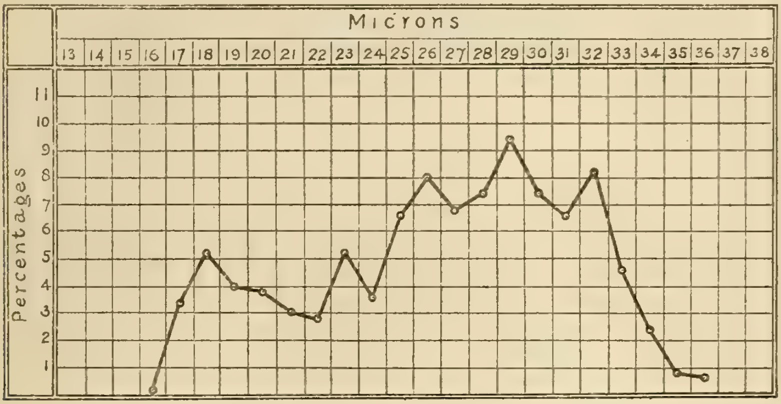


Chart 8.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain III, Chituluka, taken on nine consecutive days from Rat 953.



These last two curves from different rats also closely resemble each other. It is curious and striking that the same strain of trypanosome growing in two different animals should show this remarkable similarity.

Table VIII.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain III, Chituluka.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
August 2	953	Rat	4
" 3	953	"	6
" 6	953	"	3
" 7	953	"	8
" 8	953	"	6
" 9	953	"	13
" 10	953	"	32
		Average	10·3

IV. *Morphology of Strain IV, Chipochola.*

The following table gives the average length of this trypanosome as found in goats, monkeys, dogs and rats, 1000 trypanosomes in all, and also the length of the longest and shortest:—

Table IX.—Measurements of the Length of the Trypanosome of Strain IV, Chipochola.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	22·5	34·0	15·0

The average length of the trypanosome of Strain IV, in different species of animals, is as follows:—

Table X.

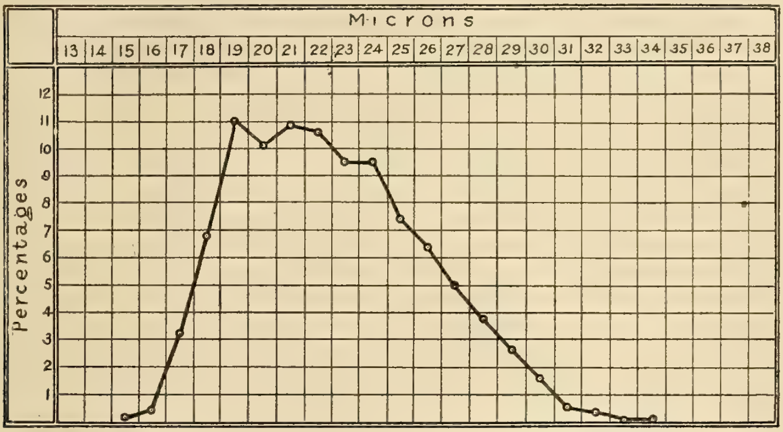
Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Goat	80	20·4	29·0	15·0
Monkey	160	22·0	34·0	16·0
Dog	260	20·9	31·0	15·0
Rat	500	22·5	34·0	15·0

Table XI.—Distribution in respect to Length of 1000 Individuals of the Trypanosome of Strain IV, Chipochola.

	In microns.									
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.
Total	2	4	32	68	110	101	109	106	95	95
Percentages	0·2	0·4	3·2	6·8	11·0	10·1	10·9	10·6	9·5	9·5

	In microns.									
	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.
Total	74	64	50	38	26	16	5	3	1	1
Percentages	7·4	6·4	5·0	3·8	2·6	1·6	0·5	0·3	0·1	0·1

Chart 9.—Curve representing the Distribution, by Percentages, in respect to Length, of 1000 Individuals of the Trypanosome of Strain II, Chipochola.



This curve is made up of 80 specimens of trypanosomes taken from the goat, 160 from the monkey, 260 from the dog, and 500 from the rat.

Chart 10.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain IV, Chipochola, taken on nine consecutive days from Rat 1337.

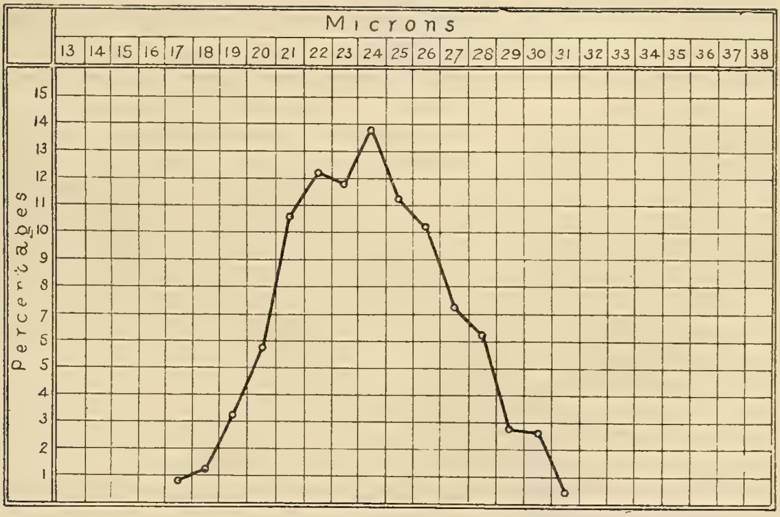


Table XII.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain IV, Chipochola.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
Sept. 20	1337	Rat	1
„ 23	1337	„	1
„ 24	1337	„	2
„ 25	1337	„	4
„ 26	1337	„	0
„ 28	1337	„	0
„ 29	1337	„	1
„ 30	1337	„	14
Oct. 1	1337	„	5
„ 2	1337	„	5
		Average	3 ·3

V. *Morphology of Strain V, Chibibi.*

The following table gives the average length of this trypanosome as found in goats, monkeys, dogs and rats, 1000 in all, and also the length of the longest and shortest :—

Table XIII.—Measurements of the Length of the Trypanosome of Strain V, Chibibi.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	22 ·4	37 ·0	15 ·0

The average length of the trypanosome of Strain V, in different species of animals, is as follows :—

Table XIV.

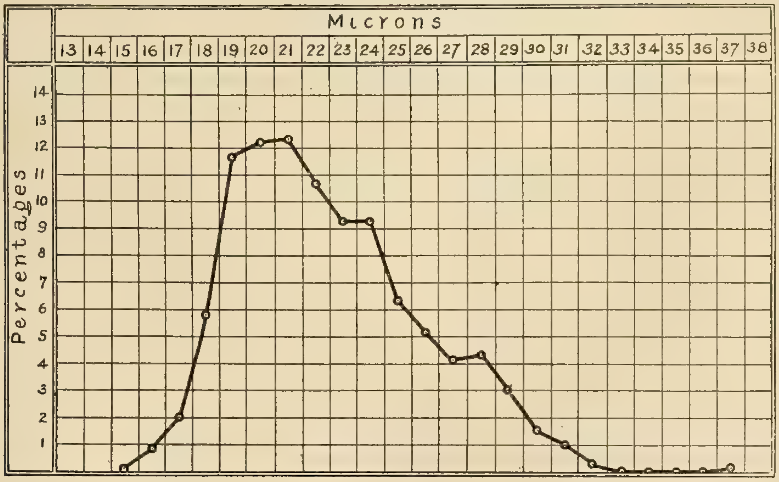
Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Goat	80	19 ·9	31 ·0	16 ·0
Monkey	160	21 ·8	32 ·0	15 ·0
Dog	260	20 ·6	37 ·0	16 ·0
Rat	500	24 ·0	32 ·0	18 ·0

Table XV.—Distribution in respect to Length of 1000 Individuals of the Trypanosome of Strain V, Chibibi.

	In microns.										
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.
Total	1	8	20	58	117	122	123	107	93	93	63
Percentages	0·1	0·8	2·0	5·8	11·7	12·2	12·3	10·7	9·3	9·3	6·3

	In microns.											
	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.	37.
Total	51	41	43	30	16	10	3	—	—	—	—	1
Percentages	5·1	4·1	4·3	3·0	1·6	1·0	0·3	—	—	—	—	0·1

Chart 11.—Curve representing the Distribution, by Percentages, in respect to Length, of 1000 Individuals of the Trypanosome of Strain V, Chibibi.



This curve is made up of 80 specimens of trypanosomes taken from the goat, 160 from the monkey, 260 from the dog, and 500 from the rat.

CHART 12.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain V, Chibibi, taken on nine consecutive days from Rat 1660.

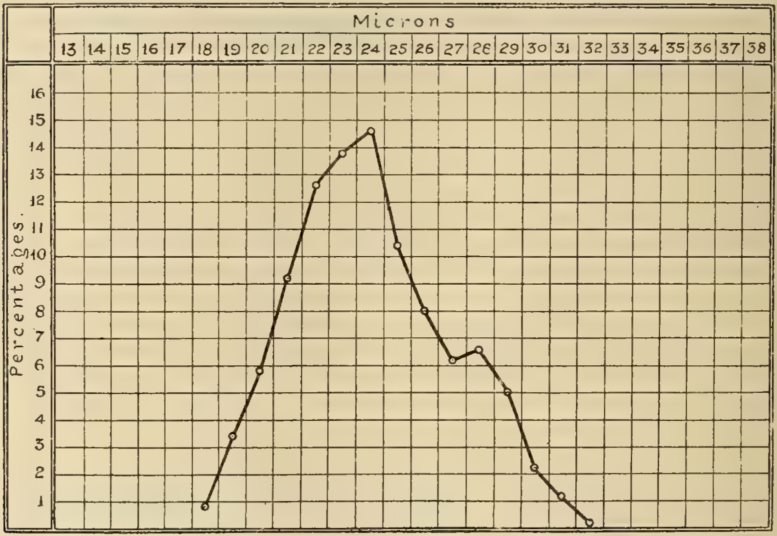


Table XVI.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain V, Chibibi.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
Dec. 3	1660	Rat	0
„ 4	1660	„	34
„ 5	1660	„	2
„ 8	1660	„	6
„ 9	1660	„	8
„ 10	1660	„	23
„ 11	1660	„	31
„ 12	1660	„	28
„ 13	1660	„	27
„ 14	1660	„	32
		Average	21·1

Comparison of the Human Strains.

The following table gives the average length of this trypanosome in the five human strains under consideration, as found in goats, sheep, monkeys, dogs and rats, 6200 trypanosomes in all, and also the length of the longest and shortest:—

Table XVII.—Measurements of the Length of the Trypanosomes of the five Human Strains. The trypanosomes have been taken from various animals.

Date.	Strain.	Name.	Number of trypanosomes.	Animals.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912	I	Mkanyanga	1220	Various	24·1	36·0	14·0
1912	II	E——	1500	”	22·2	36·0	15·0
1912	III	Chituluka	1500	”	26·1	38·0	15·0
1912	IV	Chipochola	1000	”	22·5	34·0	15·0
1912	V	Chibibi	1000	”	22·4	37·0	15·0
			6220		23·5	38·0	14·0

It must be acknowledged that, in spite of the fairly large number of trypanosomes measured, there is a marked difference in the average length of the five human strains—from 22·2 to 26·1 microns. Strains II, IV and V are similar, varying only from 22·2 to 22·5.

This difference in average length may be due to slight variations having taken place in the different strains, resulting from the passage through more or less resistant man. There is no evidence that this variation is due to treatment by atoxyl or other drugs. It has been shown that the same strain grown in two animals of the same species gives like results.

Table XVIII.—Measurements of the Length of the Trypanosomes of the five Human Strains. The trypanosomes have been taken from the rat alone.

Date.	Strain.	Name.	Number of trypanosomes.	Animal.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912	I	Mkanyanga	600	Rat	25·1	35·0	16·0
1912	II	E——	1000	”	23·1	32·0	17·0
1912	III	Chituluka	1000	”	26·4	38·0	15·0
1912	IV	Chipochola	500	”	22·5	34·0	15·0
1912	V	Chibibi	500	”	24·0	32·0	18·0
			3600		24·2	38·0	15·0

Comparison of the Curves from the Five Human Strains.

It must also be confessed that, on comparing the five curves one with another, they do not give as much assistance in classifying this species of trypanosome as was hoped. Curves I and III are alike, and coincide with that prepared by Dr. Stephens from the case of Armstrong in Liverpool, whereas Curves II, IV and V approach more to the type described by Kinghorn and Yorke from the Luangwa Valley.

Table XIX.—Distribution in respect to Length of 6220 Individuals of the five Human Strains. The trypanosomes have been taken at random from various animals.

	In microns.											
	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.
Strains I–V	1	10	41	154	325	494	528	577	512	525	511	464
Percentages	—	0·2	0·7	2·5	5·3	8·0	8·4	9·3	8·3	8·4	8·3	7·5

	In microns.												
	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.	37.	38.
Strains I–V	425	372	347	307	198	167	123	77	36	12	11	2	1
Percentages	6·8	6·0	5·6	4·9	3·1	2·7	2·0	1·0	0·6	0·2	0·2	—	—

Chart 13.—Curve representing the Distribution, by Percentages, in respect to Length, of 6220 Individuals of the Human Strain of the Trypanosome causing Disease in Man in Nyasaland. The trypanosomes have been taken from various animals.

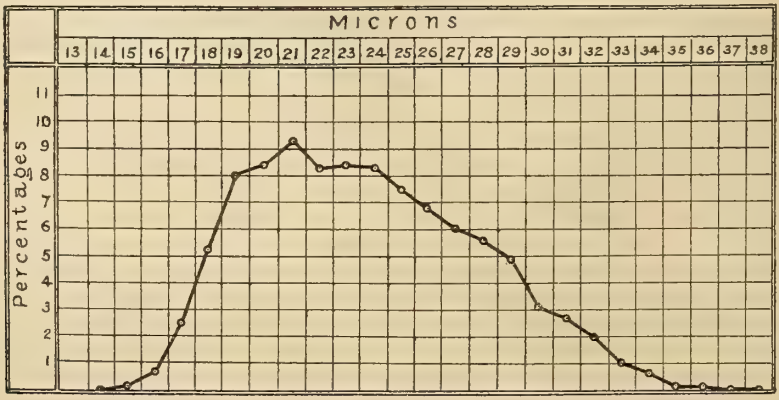
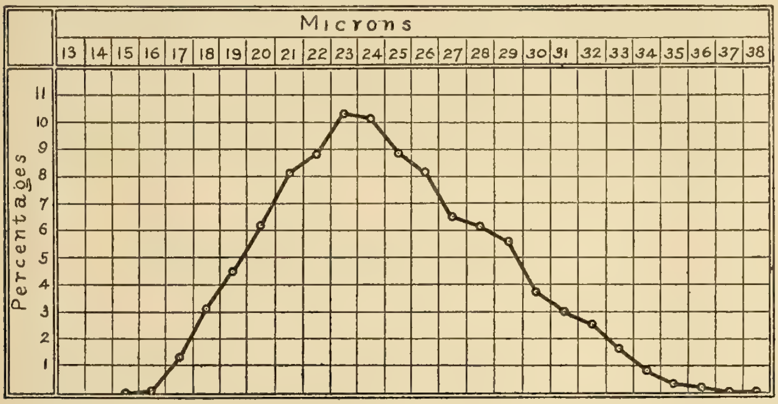


Chart 14.—Curve representing the Distribution, by Percentages, in respect to Length, of 3600 Individuals of the Human Strain of the Trypanosome causing Disease in Man in Nyasaland, taken from the rat alone.



Curves 13 and 14 will be found of use when the human strain of this species of trypanosome is compared with the Wild Game and the Wild *Glossina morsitans* strains.

Table XX.—Comparison of the Percentages of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Human Strain.

Experiment No.	Strain.	Name.	Animal.	Percentage among short and stumpy forms.
—	I	Mkanyanga	Rat	34·1
728	II	E——	,,	9·3
953	III	Chituluka	,,	10·3
1337	IV	Chipochola	,,	3·3
1660	V	Chibibi	,,	32·0
			Average	17·8

It is to be noted that in the human strain the percentage of posterior-nuclear forms varies greatly, although the method of enumeration is the same in each case. This presence of posterior-nuclear forms would have been accepted a few months ago as sufficient proof that the species dealt with was *T. rhodesiense.* Since then posterior-nuclear forms have been reported as occurring in *T. brucei* from Egypt, Uganda and Zululand. In a strain lately obtained by Theiler from the same spot in Zululand where this species was originally discovered in 1894, this percentage rose to the highest yet recorded.

Conclusions.

1. The five human strains of this trypanosome, isolated from four natives in Nyasaland and one European in Portuguese East Africa, belong to the same species.

2. This species is *T. rhodesiense* (Stephens and Fantham).

3. Evidence is accumulating that *T. rhodesiense* and *T. brucei* (Plimmer and Bradford) are identical.

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*Contributions to the Biochemistry of Growth.**—*The Glycogen-content of the Liver of Rats bearing Malignant New Growths.*

By W. Cramer and Jas. Lochhead.

(Communicated by Prof. E. A. Schäfer, F.R.S. Received January 16,—Read February 20, 1913.)

(From the Physiology Department, University of Edinburgh, and the Imperial Cancer Research Fund, London.)

In previous papers by one of us (1, 2) observations on the gaseous metabolism and on the nitrogen metabolism of tumour-bearing rats have been recorded. The present paper contains observations on the carbohydrate metabolism of tumour-bearing rats. The tumour-strain employed in our previous work was also used for these experiments; it is a spindle-celled sarcoma (J.R.S. of the Imperial Cancer Research Fund) which has a rapid growth. This tumour does not contain any glycogen. A large number of glycogen estimations were carried out with the liver of normal and of tumour-bearing animals. The glycogen estimations were made by Pflüger's method; the glucose obtained by the hydrolysis of glycogen was estimated in the first part of the work gravimetrically according to the technique used by Pflüger; later on, Bertrand's method of titration was employed. It is necessary to bear in mind that only 4 to 6 grm. of liver tissue are available for analysis in these estimations, so that with a glycogen percentage below 0·1 to 0·2 per cent. the absolute amount of glycogen present in the whole liver of a rat is so small that it cannot be determined accurately by these methods. Quantities of glycogen below this limit are therefore indicated in the following tables by the expression "$< 0·2$ per cent."

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* This research is in continuation of papers in 'Roy. Soc. Proc.,' 1908, B, vol. 80, p. 263; 1910, vol. 82, p. 307; *ibid.*, p. 316.

Since the glycogen store of the liver is known to be dependent upon and influenced by external conditions, an attempt was made to equalise these as far as possible. All the animals were of about the same age and weight; they were kept on a constant diet of bread and milk in the special metabolism cages devised by Professor Schäfer.* In some series the supply of food was limited to a definite quantity, which was sufficient to cover the requirements of the animal with regard to the growth of both the host and the tumour; in other series the supply was *ad libitum*.

The results of these first observations, which have been grouped together in Table I, show that the glycogen-content of the liver of both normal and tumour-bearing animals varies within very wide limits.

The following data were also known, but since they were found to have no relation to the variations observed in this series, they have been omitted from the table:—sex of host, weight of host. The fact that the observations extended over several years also excludes the possibility of seasonal changes on the part of the host and of cyclical changes on the part of the tumour-cells as being responsible for these wide fluctuations. These must have been caused, therefore, by factors which had not been controlled by the conditions under which the observations had been carried out.

A closer analysis of the results appeared to give a clue as to the nature of these factors. It will be seen that the variations in the glycogen-content of the liver are, as a rule, not so marked in animals killed on the same day. Now when several estimations were carried out on the same day, the animals were killed at practically the same time. Since all the animals were always fed at the same time, it seemed possible that the influence of the time which elapsed between the last meal of the animal and the moment when the liver was subjected to analysis was operative within narrower limits—in such small animals as rats at any rate—than the data given in the literature for larger animals would lead one to suppose.

Some estimations carried out with normal rats weighing about 100 grm. showed that the glycogen-content of the liver was always relatively high three to five hours after a meal, and that 15 hours after a meal it fell so low (below 0·2 per cent.) that with the small amount of liver available it could not be determined.

In order to be able to compare the conditions in tumour-bearing animals with those in normal animals it was therefore necessary to compare animals in the same state of digestion and assimilation. This object was attained in

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* For a detailed description, see 'Quart. Journ. Exper. Physiol.,' 1912, vol. 5, p. 204.

Table I.

Date.	Normal rats.		Tumour rats.			
	Weight of liver.	Liver glycogen.	Days of growth.	Weight of tumour.	Weight of liver.	Liver glycogen.
	grm.	per cent.		grm.	grm.	per cent.
20.7.10 ...			9	1·3	4·5	3·8
			9	1·3	4·5	5·5
26.7.10 ...			15	3·5	5·1	0·4
			15	9·5	5·1	1·4
28.7.10 ...	4·6	2·7				
	4·8	3·4				
	4·7	3·0				
29.7.10 ...			18	6·5	5·1	0·5
			18	3·7	4·2	0·97
31.7.10 ...			20	8·0	6·7	0·44
			20	5·7	6·4	0·36
8.11.10 ...			9	0·5	4·3	0·7
			9	0·5	5·2	5·7
12.11.10 ...	4·7	1·0				
18.11.10 ...			19	14·4	5·2	2·1
			19	10·4	6·4	1·6
22.11.10 ...			23	19·3	5·7	1·9
			23	19·8	5·6	0·8
28.11.10 ...	4·9	2·2				
	5·5	1·0				
1.12.10 ...	5·3	1·1	32	6·3	5·7	1·7
7.12.10 ...			15	7·5	4·8	0·5
			15	7·2	5·1	0·1
8.12.10 ...	3·8	0·3				
	3·8	0·4				
12.12.10 ...			20	16·5	4·5	1·5
			20	16·1	5·0	4·1
14.12.10 ...	4·7	1·14	22	3·5	6·3	1·8
	4·9	2·19	22	9·1	5·1	1·5
20.7.11 ...	4·5	1·0	10	1·0	4·2	0·34
25.7.11 ...	4·7	1·4	15	1·4	5·1	2·65
27.7.11 ...	5·1	1·0				
1.8.11 ...	5·1	1·4	22	1·6	5·0	1·6
4.8.11 ...	4·7	2·6	25	0·8	4·5	4·9
	8·9*	2·15				

* Pregnant animal.

the following manner: Animals of approximately the same weight (100 grm.) were kept for at least two weeks on a diet of bread and milk. During the last five days the amount of food eaten was determined and, unless otherwise stated, the food was supplied *ad libitum* and was kept in the cages all the time, so that the animals could feed whenever they were hungry. As a rule the amount of food consumed in 24 hours did not vary very much. In the mornings when only a little food was left at the bottom of the beaker which the animal could not reach without difficulty, fresh food was placed in the cages, so that as a rule the animals would begin to feed at once. Having determined the amount eaten, animals were selected which had consumed about equal quantities, the food was removed from the cage and a given time afterwards the animals were killed by breaking the neck. In every case a normal and a tumour-bearing animal were killed at the same time.

The results obtained are given in Table II.

Table II.

Hours after last meal.	Tumour rats.			Normal rats.		Diet.
	Weight of tumour.	Weight of liver.	Liver glycogen.	Weight of liver.	Liver glycogen.	
	grm.	grm.	per cent.	grm.	per cent.	
3	6·5	6·3	0·62	3·7	0·66	Meat.
	5·5	7·4	1·46	5·8	1·68	Bread and milk.
	9·2	4·7	1·81	4·4	3·58	Bread and milk, fasting 24 hrs. previous to last meal.
7	9·5	5·0	<0·2	4·5	0·51	Bread and milk.
	5·0	4·2	<0·2	4·5	2·43	,, ,,
	9·5	5·0	<0·2	4·8	2·24	,, ,,
				4·5	1·52	,, ,,
17	4·0	6·7	<0·2	4·8	<0·2	Meat.
24	12·0	4·7	<0·2	3·8	<0·2	Bread and milk.
	4·2	4·0	<0·2	2·6	<0·2	,, ,,

The results show that a definite difference exists between the glycogen metabolism of a normal and a tumour-bearing animal: the glycogen disappears more rapidly from the liver of a tumour-bearing rat than from the liver of a normal rat.

This result is in complete agreement with some observations on the gaseous metabolism, which were carried out by one of us in 1908 (1), and which have been confirmed since by Chisholm (3). It was found then that after a meal a tumour-bearing animal returns to the fasting state more rapidly than a normal animal.

These observations, taken together with those made four years ago by Cramer and Pringle on the nitrogen metabolism of tumour-bearing rats. throw some light on the metabolic conditions of an animal in which processes of active growth are taking place. Cramer and Pringle found that a tumour transplanted into young growing rats grows to a considerable size without interfering with the growth of the animal. As the tumour grew the nitrogen excretion of the host fell, so that there was a "sparing" of the protein metabolism. The present observations show that this "sparing" is associated with an increased carbohydrate metabolism.

In these experiments on the nitrogen metabolism it was necessary to maintain the animals on a constant nitrogen intake. It might be argued that the "sparing" of the protein metabolism takes place only with a restricted food supply, but that with an unrestricted supply of food the animals simply take in more food and thus cover the requirements of the tumour. But the experiments of Medigreceanu (4), which we are able to confirm from our own observations, have shown that, even with an unrestricted food supply, tumour-bearing animals do not eat more than normal animals. He also found that the growth of the tumour, while it does not stimulate the host to an increased intake of food, leads to an increase in the weight of the liver (5). It is interesting to note that the same holds good also for pregnant animals.

The sparing action on the protein metabolism referred to above might be explained simply by assuming that in tumour-bearing rats more carbohydrate material is burnt up instead of protein material, and that the glycogen which disappears so rapidly from the liver of tumour-bearing animals, by being used as a source of energy, protects an isodynamic equivalent of protein material, which then becomes available for the formation of new protoplasm. In that case the part taken by carbohydrates would not be specific and could be taken equally well by fats. On the other hand there is the possibility that the more rapid disappearance of glycogen from the liver is due to the fact that, in addition to the carbohydrate material which is burnt up and used as a source of energy, some glycogen is used together with nitrogenous substances as material for the synthesis of new protoplasm.

The results of the observations on gaseous metabolism by Cramer and by Chisholm indicate that the latter alternative is the correct one. For if in tumour-bearing animals more carbohydrate were oxidised in place of proteins than in normal animals, one would expect to find that the rise of the respiratory quotient from about 0·7 to about 1 which takes place after a meal rich in carbohydrates should be more persistent in tumour-bearing animals than in normal animals. But the observations which we have quoted show that this is not the case; on the contrary, if there is any difference between tumour-bearing animals and normal animals it tends, in the case of rats, to be in the direction of a less persistent rise of the respiratory quotient in the tumour-bearing animals.

We have repeatedly pointed out the close analogy which exists between the metabolic conditions of pregnancy and those of an animal bearing a transplanted new growth. In the former, where a definite organ—the placenta—presides over the nutrition of the fœtus, we have been able to show that carbohydrates are used as a material for the building up of the fœtal protoplasm. The relation of the liver to the nutrition of the tumour is not so intimate as that of the placenta to the nutrition of the fœtus and does not afford so direct an insight into this aspect of the problem. But the evidence which we have brought forward in the present paper points to the same conclusions.

Conclusions.

Glycogen disappears more rapidly from the liver of tumour-bearing rats than from the liver of a normal rat. Since observations on the gaseous metabolism showed that there is no increased oxidation of carbohydrate material in tumour-bearing animals, the results confirm the conclusion arrived at previously from observations on pregnant animals, that in growth carbohydrate material is used for the synthesis of protoplasm.

The expenses of this research were defrayed by grants from the Moray Fund of the University of Edinburgh.

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The Formation of the Anthocyan Pigments of Plants.
Part IV.*—*The Chromogens.*

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The object of the series of communications of which the present paper forms a part is the elucidation of the biochemistry and genetics of flower-pigmentation. In order to achieve this object it is necessary, in the first place, to ascertain the nature of the chemical processes which determine the formation of the anthocyan pigments, and, in the second place, to discover the chemical nature of the Mendelian characters to which the several varieties of a given species owe their power of forming and breeding true to definite types of flower colour.

The history of the working hypothesis which we use in these investigations has been summarised in an earlier paper.† This hypothesis may be expressed in the form of the equations A and B:—

$$\text{A.}\quad \underset{\substack{\vdots \\ \text{? a glucoside.}}}{\text{Prochromogen}} + \underset{\substack{\vdots \\ \text{? emulsin.}}}{\text{enzyme}} = \text{chromogen.}$$

$$\text{B.}\quad \text{Chromogen} + \underbrace{\text{oxydase}}_{\text{Peroxydase + organic peroxide.}} = \text{anthocyan pigment.}$$

Our previous and present communications are concerned with the latter, consideration of equation A being reserved for a subsequent occasion.

The position to which our previous work has led us may be summarised thus:—

The presence of oxydase in flowers may be demonstrated by means of benzidine, α-naphthol, or similar "artificial chromogens," which, when acted on by oxydase, yield pigments. These reagents serve not only to demonstrate the occurrence but also to determine the distribution of the oxydases of the flower. By the application of the method it is found that the distribution of oxydase coincides with that of anthocyan pigment. In white flowers oxydase may be present in an active or an inhibited state. In the former case some other part of the pigment-forming mechanism is absent from the flower; in the latter, the whole of that mechanism is present, but its action is prevented by the inhibition of the oxydase.

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* Previous papers of the series, which did not bear the general title, are:—

[Part I.]—"The Distribution of Oxydases in Plants and their *Rôle* in the Formation of Pigments," 'Roy. Soc. Proc.,' 1912, B, vol. 85; [Part II.]—"The Oxydases of *Cytisus Adami*," 'Roy. Soc. Proc.,' 1912, B, vol. 85; [Part III.]—"The *Rôle* of Oxydases in the Formation of Anthocyan Pigments of Plants," 'Journ. Genetics,' Nov. 1912, vol. 2, No. 3.

† "The *Rôle* of Oxydases in the Formation of Anthocyan Pigments of Plants," 'Journ. Genetics,' 1912, vol. 2, No. 3.

The present communication and that which follows (Part V) deal primarily with the chromogens of the flower.

Our first definite results demonstrating the existence of chromogens in the flower and the relation of these colourless substances with the anthocyan pigments were obtained with Brompton Stocks (*Matthiola incana*). These plants occur in numerous colour-varieties, the chief of which are pink, red, purple, and purple flaked with white.

Flowers of any of these varieties, when treated with alcohol, lose their colour rapidly. It is therefore easy to obtain a series of colourless petals derived severally from each of the colour-varieties.

If the decolorised petals of such a series be placed in water at room temperature they begin almost at once to regain their colour, and, after a quarter of an hour, each petal is found to be possessed of the identical colour of the variety to which it belongs. The petal originally pink recovers its pink colour, that from a red or purple variety becomes again red or purple, and that from a white-striped purple variety reproduces with faithful accuracy the purple and white pattern of the original.

Despite the fact that our experiments have been carried out during the winter months, when suitable material is somewhat scanty, we have been able to prove that a similar recovery of natural colour is exhibited by many other flowers, for example :—

Aubretia, wallflower (Cruciferæ).
Viola (Violaceæ).
Pelargonium (Geraniaceæ).
Cyclamen, polyanthus (Primulaceæ).
Begonia (Begoniaceæ).
Azalea (Ericaceæ).
Bilbergia (Bromeliaceæ).
Dendrobium (Orchidaceæ).

Recovery of colour is shown also by the vegetative parts of plants which contain anthocyan, for instance, the leaves of Fuchsia and Tradescantia, and the fronds of the Royal fern (*Osmunda regalis*). Loss and recovery of colour are therefore phenomena of very general occurrence, and may be regarded as characteristic of many, if not of all, kinds of anthocyan pigments.

The wallflower is of special interest in this connection, in that the brown varieties with which we have worked contain representatives of the two types of pigment—anthocyanic and plastid-derived pigments—to either or both of which the colour of flowers may be due. The brown colour of the wallflower is produced by a purple anthocyan pigment and a yellow plast pigment. When acted on by alcohol a brown petal becomes decolorised; but it recovers to a purple colour when treated with water. The recovery to purple instead of brown is due to the fact that the yellow plastid-pigment which contributes to the original brown colour is soluble in alcohol and is therefore extracted from the tissues by this reagent. Thus only the colourless antecedent of the purple anthocyan pigment is left in the cells. Treated with water that antecedent gives rise to a purple pigment which, since it is no longer mixed with yellow, produces its proper optical effect. The yellow pigment may be obtained free from the anthocyan pigment by evaporating the alcoholic solution and washing the residue with water, in which the plast pigment is insoluble. The power of recovering to the original colour serves as a means of distinguishing the pigments of the anthocyan class from those which are derived from the plastids.

The reproduction of the original colour in the petals of stocks and other plants is open to two alternative interpretations. On the one hand, it may be regarded as a phenomenon of like nature to that exhibited by indicators; on the other hand it may be attributed to the oxidation of a chromogen.

Immediate choice between the two interpretations is rendered difficult by reason of the fact that acids and alkalis exercise marked and definite effects on the colours of the anthocyan pigments contained in the flowers. Thus, in the presence of alkalis the pigment in the petals of stocks assumes a green-blue colour and in the presence of acids it becomes pink. Moreover the chromogen extracted by means of 50-per-cent. alcohol from the petals of stocks behaves as a very sensitive indicator. Dilution of the alcoholic extract with ordinary distilled water—which contains carbon dioxide—suffices to produce a pale pink colour. With mineral acids the colour becomes intense and with alkalis it passes through blue and blue-green to green.

Nevertheless, and in spite of the complication introduced by these indicator effects, the evidence of the experiments now to be described points very definitely to the conclusion that the indicator hypothesis must be discarded in favour of its alternative.

The petals of stocks decolorised by strong alcohol contain oxydase. The reproduction of the original colour by the immersion of decolorised petals in water is hastened by the addition of hydrogen peroxide. When a pink and a purple petal decolorised in the same alcohol are transferred to water to which a drop or two of hydrogen peroxide is added, the petals recover pink and purple respectively. Therefore it follows that the activating action of the peroxide is due to its provision of oxygen and not to its acidity.

In water which has been boiled for a long time in order to remove the oxygen, the recovery of colour, if it take place at all, occurs more slowly than in unboiled water. The addition of dilute hydrogen cyanide—a substance known to inhibit oxydase-action—prevents the recovery of colour. The results of other experiments designed to decide between the indicator and oxidation hypotheses lend further support to the latter. Thus decolorised petals in which the pigment has been caused to reform may be again decolorised either by leaving them in water till the pigment has diffused away or by transferring them to alcohol. Petals treated in this manner, if placed in hot water, produce once again their natural pigments.

Again, the restoration of pink colour to decolorised petals of a pink variety may be brought about in an alkaline medium; for example by transferring the petals from alcohol to water containing a small quantity of hydrogen peroxide which has been rendered faintly alkaline. The pink colour which is thus induced changes subsequently to purple. Conversely the purple colour returns to a petal of a purple variety even though the medium in which the change is effected be acid. In this case the recovered purple soon becomes pink owing to the action of the acid.

The last experiment is rendered still more conclusive if the procedure be modified in the following manner. Petals of purple stocks are incubated with 99-per-cent. alcohol to which enough citric acid has been added to render the alcohol distinctly acid to litmus. The petals became almost decolorised, retaining only a faint pink colour. When transferred to distilled water—which is not acid to litmus—pigment is produced in considerable quantities and the colour of the pigment is at first red but soon becomes purple. If the colour were of the type of an indicator reaction the effect of the water would be not to intensify but to dilute the tint.

The conclusion which we have reached as the result of these observations is that, although indicator changes run parallel with the changes involved in the formation of anthocyanic pigments, the latter arise as the result of the oxidation of chromogens.

It remains to mention the remarkable acceleratory effect which high temperature has on the reproduction of the natural pigments of stocks. As stated already, a petal recovers its colour in water at room temperature in the space of a quarter of an hour. At higher temperatures the recovery is more rapid and if a petal be dropped into water which has been heated to near the boiling point the recovery of colour is almost instantaneous.

We turn now to the detailed interpretation of the facts of loss and recovery of colour; and we deal first with the loss of colour which takes place when petals are dehydrated.

The evidence about to be given supports the conclusion that the loss of colour is due to the action of a reducing agent. In the present state of our knowledge of the reducing processes which occur in the cells of plants it is not possible to affirm that the agents of these processes are of the nature of specific catalysts. We propose therefore to avoid using the word reductase and to employ the indifferent term "reducing agent" in the description of the phenomena of decolorisation.

A careful examination of the petals of stocks subjected to the action of alcohol makes it difficult to escape from the conclusion that decolorisation is due to the activity of a reducing agent. It is easy to demonstrate that the loss of colour is not due merely to a dissolution of the pigment and its diffusion throughout a large bulk of fluid.

As evidence that the loss of colour is due to the action of a reducing agent we may cite the following facts:—

The immersion of the petals in alcohol produces three immediate effects,—a rapid evolution of gas, a reduction in the amount of colour, and a discharge from the petals of a certain amount of pigment which dissolving in the alcohol gives rise to a marked coloration of that reagent.

Similar effects are produced, but more rapidly, if previously to their immersion in alcohol the petals are treated for about half a minute with chloroform.

As a consequence of the discharge of the pigment the alcohol becomes deeply coloured—purple or red according to the variety of stock used in the experiment. If the alcohol be decanted at once its colour disappears with remarkable rapidity and in less than 5 minutes the liquid becomes colourless or at most faintly coloured. The partly decolorised petals, from which the first lot of alcohol was removed, if treated with more of this reagent, undergo further decolorisation, but at a much slower rate. The simultaneous evolution of gas and the discoloration suggest that the effect of the alcohol is to liberate a reducing agent which brings about the deoxidation of pigment and an evolution of oxygen. Further evidence of the presence of such a reducing agent is provided by extracts prepared by pounding fresh petals with alcohol. The colour of the extract is at first identical with that of the petals from which it was made; but sooner or later the colour fades and the solution becomes colourless. The fading is rapid in concentrated alcohol and slow in alcohols of somewhat weaker grades. The agent responsible for the fading is resistant to high temperatures. Thus if alcoholic extracts be evaporated to dryness and the residues be taken up with water, the fading of the solutions still takes place Further evidence in favour of the view that decolorisation is due to reduction is offered by the results of experiments on the effect of extracts in inhibiting and in reversing oxydase-action.

The experiments were made in the following ways:—

1. *Extracts made from Stocks by Grinding the Petals with Alcohol.*—A solution of the peroxydase of bran is rendered of such a strength that it just gives the characteristic blue reaction with benzidine and hydrogen peroxide. Petals of a coloured variety of stock are ground with alcohol, the extract is evaporated to dryness, and the residue dissolved in water. If a few drops of the latter solution be added to the solution of peroxydase, and if the benzidine-hydrogen peroxide test be applied, no colour-reaction ensues. The oxydase is prevented by the reducing agent from bringing about the oxidation of benzidine. Only if it be increased very considerably in amount is the oxydase able to overcome the opposing influence of the reducing agent, and to bring about the oxidation of the benzidine.

2. *Extracts obtained by Immersing Intact Petals in Strong Alcohol.*—The use of extracts made by grinding petals with strong alcohol is open to obvious objections. We have, therefore, used extracts obtained by the immersion of intact petals in strong alcohol.

For this purpose petals of purple stocks are immersed in alcohol of 99 per cent. When the alcohol is decanted from the tube containing the petals, its colour (pale purple) disappears in the course of a few minutes. On evaporation over a water-bath it yields a purple residue. For the purposes of control an equal volume of alcohol of the same strength as that used for the extraction of the petals is also evaporated to dryness. A bran peroxydase is prepared of such a strength that when a given volume of it is added to a given volume of a weak solution of benzidine containing one drop of hydrogen peroxide a definite but pale blue colour is produced. The addition of similar volumes of peroxydase, hydrogen peroxide and benzidine to the purple residue results in the production of no blue colour, whereas the colour develops normally when the reagents are added to the vessel in which the alcohol alone has been evaporated to dryness. The alcohol which has been in contact with the petals, like the alcoholic extract obtained by maceration, prevents the action of oxydase.

Yet more conclusive is the result when the blue solution, produced by the action of bran peroxydase and hydrogen peroxide on benzidine, is added to the residue obtained by evaporating alcohol which has been in contact with intact petals. The blue colour of the former is discharged immediately, that is to say, the action of the oxydase is reversed, and the blue product of the oxidation of benzidine is reduced to its original colourless state. That this effect is not due to reducing agents present in the alcohol is shown by the fact that no discharge of colour is brought about by the addition of the blue oxydase-benzidine solution to the residue left after the evaporation of alcohol which has not been in contact with petals. This method of demonstrating the presence of a reducing agent is the more conclusive in that whereas alcohol alone reduces oxydase-activity, it does not bring about a reversal of the action. The only effect of alcohol on the blue colour is to precipitate the blue pigment.

We have shown in a previous communication (Part III) that the oxydases of the flower not only act on the forerunners of pigment contained in the petals but also on the artificial chromogen benzidine and give rise to pigments; we now show that flowers contain reducing agents which are not only capable of inhibiting the action of oxydase, but are able also to reduce both the natural pigments of the flower and the "artificial" benzidine pigments to the colourless state of chromogens.

Two facts stand out prominently in the foregoing investigation of decolorisation. These facts are that the reducing agent is very resistant to high temperatures and that it is active in strong alcohol. The former we have studied in sufficient detail only to be able to state that the reducing agent is not destroyed by exposure to a temperature of 100° C., the latter fact has been investigated more fully and with the following results:—

Both evolution of gas and fading of the flower take place rapidly in alcohol of 95 per cent. These processes go on, albeit more slowly, in yet stronger alcohol. Thus with the ordinary absolute alcohol of the laboratory (99 per cent.) a certain amount of gas is evolved and colour begins to disappear; but when petals are placed in alcohol of approximately 100 per cent. both processes, although they take place, come to an end much sooner than in the alcohol of slightly lower grade.

We conclude, therefore, that the reducing agent which brings about decolorisation of the petals of stocks is able to exhibit its specific action in tissues which are almost completely dehydrated. We have, moreover, evidence that loss of colour occurs naturally in the plant. We know, for example, that in many plants light shades of colour are dominant to dark shades; we know also that the flowers of such plant of stocks may assume as they fade a new colour, and we know that the colour of some flowers undergoes a marked change during the course of the day. Such changes are to be ascribed to the simultaneous presence in the petals of pigments, chromogens, oxidizing and reducing agents.

We have now to consider the conditions under which recovery of colour occurs.

Evidence has been given already in favour of the interpretation that recovery is brought about by the oxidation of chromogen.

There is, however, one series of facts, namely, those bearing on recovery of colour by petals immersed in strong alcohol, which seems to throw doubt upon this conclusion.

For, as several investigators have shown, increasing concentrations of alcohol exercise a progressively adverse effect on enzyme action. Thus, Hudson (1910),* working with invertase has expressed the effects of different concentrations of alcohol in the form of a regular curve. He finds that in 70-per-cent. alcohol invertase retains only 10 per cent. of its activity.

We have studied the relation of the activity of maltase to alcoholic concentration and find that this enzyme is even more sensitive to ethyl alcohol than is invertase; the activity of maltase ceasing in 60 per cent. With methyl alcohol a 40-per-cent. solution suffices to render the enzyme inactive.

Our experiments with emulsin, which confirm those published in 1912 by Bourquelot, give results similar to the foregoing except in one important particular.

The activity of emulsin falls rapidly as the concentration of alcohol increases to 40 per cent. After this point is reached the activity falls off more slowly and some activity may be detected in solutions containing 90 per cent. of alcohol; in solutions containing from 40 to 90 per cent. of alcohol the activity of emulsin is proportional roughly to the amount of water present in the solution.

For the purpose of investigating the effect of alcohol on oxydase we have made use of the Lovibond tintometer. We measure by means of this apparatus the depth of coloration—a mixture of red and yellow—produced by the action of bran peroxydase on guaiacol.

The curve representing the amount of oxydase action—as measured by the tintometer—is similar to the curves which have been obtained for invertase, maltase and emulsin. As the alcoholic strength increases the activity of oxydase falls. In 50-per-cent. solutions it becomes very small and ceases altogether in 70-per-cent. alcohol. Alcohol causes a similar retardation of the benzidine reaction; but the colour of the latter is not suitable for tintometric estimation.

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* Hudson, C. S., 'U.S. Dept. of Agric., Bureau of Chemistry, Circular 58.'

The results obtained in test-tubes are opposed to the view that the recovery of colour in petals immersed in strong alcohol is due to the activity of oxydase. General considerations, however, led us to suspect that although alcohol of 70–80 per cent. may prevent the action of the oxydase in solutions extracted from plant-tissues, it might prove less potent to retard the action of oxydases in the tissues themselves. We have confirmed the truth of this suspicion in the following way:—

Petals of purple stocks were incubated with 99-per-cent. alcohol and when decolorised they were placed, some in 70 per cent., and others in 80, 90, and 95-per-cent. alcohol. Equal quantities of a solution of benzidine in water-free alcohol were added to each tube containing the petals, one drop of hydrogen peroxide was introduced into each tube, and the preparations were placed in the incubator at 37° C. Examination of the petals after half-an-hour showed that the petal treated with 70-per-cent. alcohol gave a well marked brown benzidine reaction for oxydase, the petal in 80 per cent. showed a very distinct reaction, that in 90 per cent. an equally good or even better reaction, and that in 95 per cent. a slight but distinct reaction in the veins of the claw.

Whence it follows that the peroxydase of stock petals is capable—if peroxide be present—of bringing about the oxidation of benzidine even in a medium containing 95 per cent. alcohol; and we infer that what is true of this artificial chromogen is true of the natural anthocyanic chromogen, namely, that the latter may undergo oxidation even in the presence of 95-per-cent. alcohol.

Thus the conclusion is reached that, although the experiments with oxydase extracted from plant tissues are adverse to the view that recovery of colour is due to oxidation, the more apposite and crucial experiments with the oxydases contained within the petals lend powerful support to that view.

The series of observations described in the foregoing pages lead us to the following conclusions:—

In concentrated alcohol the anthocyan pigments are reduced to the state of colourless chromogens. The reduction is brought about by reducing agents, the nature of which is unknown. The reducing agents may be specific chemical substances; they may perhaps be of the nature of catalysts; they are probably not enzymes (reductases). It is interesting to observe that an effect similar to that exercised by the reducing agent contained in stocks is brought about by hydroquinone, though not by formaldehyde.

When the concentrated alcohol is replaced by water, the oxydases, which are not destroyed by the former reagent, resume their activity, and colourless chromogen is converted into anthocyan pigment.

The fact that the colours of the pigments thus produced are identical severally with those of the natural petals, indicates either that chromogens of more than one kind exist in the different colour-varieties of stocks, or —what for our present purpose is nearly the same thing—that one chromogen is present, and associated with it are substances which determine the coloration of the oxidised produce of the chromogen.

The petals of plants such as stocks contain much larger quantities of chromogen than are used in the natural flower. Not only may the original depth of colour be recovered, but the pigment so formed may be removed from the tissues and further instalments of pigment may be produced. Whether the reserves of chromogen contained in the flower occur as such or, as would appear more probable, in the form of prochromogen, we cannot at present say.

The factor which determines the direction in which the pigment-producing reaction shall go is the amount of active water present in the cells. As the amount of water decreases, the reducing agents of the cell become active and oxydase becomes inert; as the amount of water increases oxydase action comes into play and the reducing agents are either destroyed, or, if they persist, any action which they exert is masked by the superior and opposed activity of oxydase. The relations may be expressed diagrammatically by the following scheme:—

$$\text{Chromogen} \quad \underset{\xleftarrow{}}{\underset{\left\{\begin{matrix}-\text{Water}\\ \text{Reducing agent}\end{matrix}\right\}}{\overset{\xrightarrow{}}{\overset{\left\{\begin{matrix}\text{Oxydase}\\ +\text{Water}\end{matrix}\right\}}{}}}} \quad \text{Anthocyan pigment.}$$

The occurrence of reducing bodies side by side with oxydases in the anthocyan-containing tissue of plants, the antagonistic relation which obtains between the reducing and oxidising agents of the cell, and the relations which exist between the activities of these agents and the degree of hydration of the cell are calculated to throw light, not only on the phenomena of pigment-formation and pigment-inhibition in plants, but also on others of wider import. Following the clue offered by these experiments we may hope perhaps to advance towards an understanding of the biochemical meanings of activity and latency of seeds, of the enforced and natural awakening of vegetation, and of cognate phenomena.

A discussion of the foregoing facts in relation with these phenomena lies, however, beyond the scope of the present communication.

The Formation of the Anthocyan Pigments of Plants. Part V.—*The Chromogens of White Flowers.*[*]

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The series of communications of which the present paper forms a part (Part V) deals with the biochemistry and genetics of pigmentation in plants. Parts I and III of the series describe the *rôle* of oxydases in the formation of the anthocyan pigments of flowers; Part IV gives an account of the chromogens which constitute the colourless antecedents of these pigments; and the present paper has for its object the investigation of the chromogens in white flowers.

The subject is of interest from the point of view both of biochemistry and genetics; for, as has been discovered by Mendelian research, the white flowers which occur so commonly in cultivated and wild plants belong to more than one category.

The types of white flowers recognised hitherto are known respectively as dominant and recessive whites. As shown by Keeble and Armstrong, both dominant and recessive white flowers contain oxydase (or peroxydase). In the former the oxydase is inactive owing to the presence of an inhibitor; in the latter it is active.

Inhibition of oxydase suffices to account for the absence of colour from dominant white flowers. In order to account for the absence of colour from recessive whites it is assumed that some part of the colour-forming mechanism—for example, the chromogen—is lacking from the flowers. It is, however, possible that lack of colour may be in some cases the consequence not of absence of an essential constituent of the colour-producing mechanism, but of the failure of these constituents—all of which are present in the flower—to come together and interact with one another.

The results of experiments about to be described show that both kinds of recessive white flowers exist.

As the result of treating the white flowers of *Lychnis coronaria* var. *alba* with alcohol (15–80 per cent.), chloroform, ether, or carbon disulphide, a brown pigment develops.

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[*] Parts I and II, Keeble and Armstrong, 'Roy. Soc. Proc.,' 1912, B, vol. 85, pp. 214 and 460; Part III, Keeble and Armstrong, 'Journal of Genetics,' November, 1912; Part IV, Keeble, Armstrong, and Jones, 'Roy. Soc. Proc.,' 1913.

This pigment is at first limited to the veins, though subsequently the whole petal becomes distinctly coloured. The depth of colour is considerable, and the general aspect of the brown petals resembles closely that produced by the action of benzidine. There seems no doubt, indeed, that the brown coloration obtained by treating petals of *Lychnis coronaria* with an alcoholic solution of benzidine is due to this effect of the alcohol rather than to a reaction between oxydase and benzidine. That this is so is indicated by the fact that addition of hydrogen peroxide to a petal so treated causes a further and immediate darkening.

If, however, petals are immersed in absolute alcohol from which the water has been removed by anhydrous copper sulphate, no browning occurs. This is to be expected if the browning is due to oxydases, for, as shown in Part IV, the oxydases are thrown out of action temporarily by dry alcohol.

Petals transferred to water after soaking an hour or so in dry alcohol rapidly develop the brown colour; but petals that have been left several days in the dry alcohol form no brown pigment on transference to water, nor does the addition of hydrogen peroxide cause it to appear. If now it be assumed that the formation of the brown pigment under the influence of chloroform, alcohol, etc., is due to interaction between a colourless "chromogen" and an oxydase (kept apart in the intact petal but allowed to come together when the alcohol has destroyed the impermeability of the plasmatic membrane); then the failure of the pigment to develop in the case of petals that have been soaking some time in alcohol may be taken to indicate that the chromogen has been removed from the petals and diffused out into the alcohol.

Failure of the brown colour to appear is not due to the destruction of the body that functions as peroxide, since addition of hydrogen peroxide is without effect; nor is it due to the destruction of the peroxydase itself, since the petal, after long immersion in alcohol, gives a good benzidine reaction for peroxydase.

If the above view of what occurs be correct, the absolute alcohol in which the petals have been soaked should contain the chromogen in solution. In order to prove that this is the case, a considerable number (150) of *Lychnis coronaria* flowers were treated with 50-per-cent. alcohol, raised to boiling point in order to destroy the oxydase.

After concentrating the extract to a small bulk, white Lychnis flowers, soaked in dry alcohol as above to remove the chromogen, and known to contain peroxydase, were incubated at 36° C. in the solution. The flowers remained colourless while in the extract, but when they were transferred to water containing hydrogen peroxide a reddish-brown pigment appeared at once in those parts of the flower which contain peroxydase. Hence it is demonstrated that the petals of Lychnis contain a chromogen, which, when extracted from the flowers, is acted on by the peroxydase contained in the petals and gives rise to a red-brown pigment. The peroxydases of *Primula sinensis, Primula obconica, Dianthus* sp., etc., were shown also to bring about —in the presence of hydrogen peroxide—an oxidation of the chromogen extracted from the petals of Lychnis. A similar chromogen has been extracted from the white-flowered variety of *Anemone japonica.* Like that obtained from the flower of *Lychnis coronaria,* it yields pigments when acted on by the oxydases of petals of various plants.

The white flowers of *e.g. Lychnis coronaria* thus yield an extract which can be used to demonstrate the distribution of oxydases in place of a benzidine solution.

The experiments show, moreover, that these flowers of *Lychnis coronaria,* although they are white, contain both oxydase and chromogen.* It is therefore probable that these constituents are located in different cells or parts of the same cell, and that whiteness is due to the fact that the plant lacks the means of bringing chromogen and oxydase into contact with one another.

As has been mentioned already, the pigment obtained by the action of the peroxydase of the petals of *Lychnis coronaria* on the chromogen extracted from these petals is of a reddish-brown colour. It might, therefore, be urged that the chromogen which gives rise to this pigment is not that which in coloured flowers yields the red anthocyan pigment of the natural petals.

The objection is weighty; but that it may be met is shown by the following considerations and experiments :—

1. It is known that changes in the chemical nature of the chromogen, the degree of oxidation,† the conditions under which the reactions occur, and the presence of traces of other substances,‡ affect the colour of the end product of oxidation. Too much weight, therefore, should not be attached to mere difference in colour as the colour is very susceptible to alteration.

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* Since browning of the fresh petal occurs under the influence of alcohol alone, the body that behaves as a peroxydase towards *e.g.* α-naphthol, can behave as an oxydase towards the natural chromogen.

† In this connection it may be noted that if a pink bract of *Bilbergia* sp. be immersed in H_2O_2, the pink pigment becomes changed into brown, presumably as the result of further oxidation. In 'U.S. Dept. of Agric. Bureau of Plant Industry Bulletin,' No. 264, 1913 (received as the present paper goes to press), H. H. Bartlett records a red pigment of Dioscorea as becoming brown on oxidation.

‡ Chodat, R. "Nouvelles Recherches sur les Ferments Oxydants. Les matières protéiques et leurs dérivés en présence du réactif *p*-crésol tyrosinase." 'Arch. Sci. Phys. Nat.,' 1912 (IV), vol. 33, pp. 70, 225.

2. The behaviour of flowers of Brompton stocks, as described in detail in Part IV of this series, provides a convincing proof that it is possible for all the mechanism for colour production to be present in a flower, and yet for the bodies concerned not to interact to produce pigment until the plasmatic impermeability has been destroyed.

The fading and recovery of colour of petals of these plants was observed by the present writer during the preliminary experimental work in connection with the above paper. The facts will be referred to here only in so far as they illustrate the point at issue.

If coloured flowers of Brompton stocks are soaked in absolute alcohol, the contained pigment gradually fades; on transferring the colourless petals to water they quickly become coloured, the "recovered colour" being of exactly the same shade as that of the fresh flower used.

In the paper referred to evidence is presented that the fading of the coloured petal in alcohol is due to the reduction of the pigment to a colourless state, as well as to its diffusion out into the surrounding liquid, and that the formation of pigment when the petal is transferred to water is the result of an oxydase converting into pigment a colourless chromogen substance contained in the petals in addition to a re-oxidation of the reduced pigment remaining.

Thus, in stock, oxydase and chromogen are both present, and the conditions are naturally such as to allow a proportion of these two bodies to come together to produce pigment. In the white-flowered variety of *Lychnis coronaria* the natural conditions are never such as to allow any interaction between oxydase and chromogen. On treatment with alcohol the barrier is removed by the destruction of the plasmatic impermeability, and, as a result, pigment is produced.

The method which serves in the case of Lychnis to bring chromogen and oxydase together, and causes them to interact with one another, serves with Brompton stocks to bring about a large increase in the amount of pigment present—which pigment is of the same colour as that occurring in the flower under natural conditions.

By use of such methods the following types of white flowers have been demonstrated in the course of this investigation:—

1. *White Flowers which contain an Oxydase and a Chromogen, e.g.* Lychnis coronaria, Anemone japonica, Chrysanthemum *sp.*—When petals of these plants are subjected to the action of alcohol, chloroform, etc., a colour change is produced. The colour may be brown, as in *Anemone japonica,* and appear more or less evenly all over the petal; or of a reddish tinge, as in *Lychnis coronaria,* where the colour is located chiefly in the veins. In both these

examples the depth of colour obtained is very considerable; in the case of Chrysanthemum the colour change is only slight.

All the flowers belonging to this class give the characteristic peroxydase reaction with benzidine or α-naphthol solutions and hydrogen peroxide.

2. *White Flowers which contain a Peroxydase and a Chromogen.*—This type of white flower is illustrated by certain varieties of *Dianthus caryophyllus* (*e.g.* var. "Mrs. Sinkins,") and of *Dianthus barbatus* (Sweet William), etc.

These flowers on treatment with dilute alcohol, chloroform, etc., show no development of colour, but the addition of H_2O_2 causes a rapid formation of pigment. In many flowers of this class colour is produced only locally in the petal on the addition of hydrogen peroxide. On testing such a flower with benzidine solution with the subsequent addition of hydrogen peroxide, a peroxydase reaction is obtained only in these same localities. The peroxydase is limited, therefore, to those areas that give a colour reaction when treated with alcohol, etc., and hydrogen peroxide.* Whether the chromogen, which contributes to the reaction occurring in alcohol is present in the regions from which peroxydase is absent is as yet undetermined.

3. *White Flowers which contain a Peroxydase but no Chromogen.*—The white-flowered varieties of *Plumbago capensis* and *Swainsonia Tacsonia* illustrate a third type of white flower.

No colour reaction is given after treatment with chloroform or similar bodies even after the addition of hydrogen peroxide. Such petals, however, give in every case a reaction with benzidine and hydrogen peroxide.

4. *White Flowers which contain no Oxydase or Peroxydase.*—A fourth type may perhaps be inferred from the behaviour of a white variety of Sweet William investigated by Keeble and Armstrong, which was found to give no benzidine reaction, direct or indirect, and was therefore assumed to lack oxydase and peroxydase. The possibility of an inhibitor being present was not overlooked, but was not investigated. Information as to the occurrence of a chromogen in these flowers is also wanting.

The interpretation suggested above of the behaviour of white flowers when treated with alcohol, etc., is the most obvious and simple one, but it is fully recognised that intermediate steps may occur of which no account has been taken.

In *Lychnis coronaria*, it may be that a chromogen, as such, does not occur in the intact petals, but is split off from a body which one may term a pro-chromogen, after the plasmatic impermeability has been destroyed by treatment with alcohol.

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* In coloured varieties these same areas are often the only parts of the flower containing pigment.

A modification of the interpretation on these lines, however, does not affect the general hypothesis as to the existence of several types of white flowers, or the inference that pigment is not necessarily produced although all the requisite ingredients for the production of colour may be present.

On the Occurrence of a Ganglion in the Human Temporal Bone not hitherto Described.

By Albert A. Gray.

(Communicated by Prof. J. G. McKendrick, F.R.S. Received November 26, 1912,
—Read February 6, 1913.)

(Plate 6.)

The existence of a previously unknown nerve plexus associated with a comparatively large ganglion embedded in the substance of the human temporal bone must be regarded as a somewhat surprising fact at this period in the history of human anatomy. It may be well, therefore, to describe in a few lines the process which led to the discovery.

While making some preparations of the middle ear of animals according to my own method, I discovered the presence of a large plexus of nerves on the posterior surface of the bulla of the sheep. This plexus was found to be composed of bundles derived from the pneumo-gastric and the facial nerves. Since the preparation was only macroscopic, I was unable to ascertain whether nerve ganglion cells were present. Such a plexus has not been described in the human subject, unless the two minute bundles of fibres which pass between Arnold's nerve and the facial nerve be dignified by the name of plexus. It seemed, therefore, highly probable to me that some corresponding structure might exist in the human subject which had not hitherto been described, and a search was accordingly made.

The initial difficulty lay in the fact that this portion of the temporal bone is very different in man from that in the sheep. In the latter there is a large bulla, but no mastoid process; whereas in man there is a large mastoid process and no bulla. In man the only indication of a bulla is the little *cul-de-sac* which runs backwards from the lower, inner, and posterior corner of the tympanum. In the human subject a mass of bone fills the space which in mammals is occupied by the bulla. The plexus, therefore, if present in the human subject, would probably lie embedded in bone. The second difficulty lay in the fact that the structures in the region involved do not have the same relationship to one another in the human subject as in the sheep. The stapedius muscle, for example, lies below, internal to, and in front of the facial nerve in man, not above it as in the sheep.

The plexus which was seen in the sheep had a position a little below the lower termination of the stapedius muscle, and internal to the facial nerve. Since the stapedius muscle has, so to speak, been rotated downwards and inwards in man, as compared with the position which it occupies in the sheep, the most likely place for the plexus would be a little below the lowest point of the origin of the stapedius muscle. This clue was followed, and, as will be shown later, the inference was justified by the result.

The petrous portion of a human bone was removed in the fresh state and a small piece, including the region indicated above, was decalcified and prepared for microscopic section. The preparation, after very complete decalcification, was embedded in celloidin, and sections in the vertical plane were made from before backwards. The sections were stained with iron-alum and hæmatoxylin.

The series of sections was not complete, but it was quite sufficient to reveal the existence of a plexus, corresponding to that of the sheep, but very much smaller in size. Owing to the incompleteness of the series it cannot be definitely stated what is the exact origin of all the bundles which go to compose the plexus, but it is clear that they are derived from at least two sources: first, the facial nerve; second, the auricular branch of the pneumogastric.

While it was interesting to discover this plexus in man, a much more remarkable fact was also revealed. This was the presence of a comparatively large ganglion associated with the plexus, and, like it, embedded in bone. The first impression on finding this ganglion was that it was a portion of one of the ganglia of the glossopharyngeal or pneumogastric nerves. But closer examination showed that this could not be the case, because it was at a considerable distance from the trunks of these nerves, and, moreover, it was embedded in bone.

A further search was then made among the writer's macroscopic preparations of the temporal bone, and the ganglion was discovered to be present in it also. It is shown in Plate 6, fig. 1, which is taken from the writer's text-book on 'Diseases of the Ear.' It will be seen that the ganglion, *g.g.*, lies immediately below the inferior termination of the stapedius muscle, and about the same distance in front of the facial nerve in the vertical portion of its course. The auricular branch of the pneumogastric nerve passes upwards from the jugular fossa through the bone towards the ganglion (Plate 6, fig. 1, *a.p.*).

The general position of the ganglion having now been described, it remains to give a few details concerning its finer structure. This can only be done from a series of microscopic sections. Such a series was made, but the decalcification process was too energetic, and in some of the sections portions of the bone and even portions of the ganglion itself have been washed away.

The ganglion is very irregular in shape, and is surrounded on all sides by bone. As a result of this irregularity in shape different portions of the structure come into view in different portions of the same section, so that at first sight it would appear that there are two or more ganglia. But when the series is studied carefully it is found that this appearance is merely due to the presence of outlying semi-detached portions of one ganglion.

The name which I propose to give to the structure is "the Stapedial Ganglion." It is situated close to the lowest point of the stapedius muscle in man, and the name suggested is, perhaps, as appropriate as any.

The first section (fig. 2) passes through the anterior portion of the ganglion. Considerable portions both of the bone and of the ganglion itself have been lost in the course of preparation, but the upper and lower parts of the latter are seen, *g.g.* The posterior semicircular canal is seen to the left of the uppermost portion of the ganglion, and the jugular fossa is shown in the left lower part of the photograph. A bundle of fibres derived from Arnold's nerve runs in the direction of the ganglion.

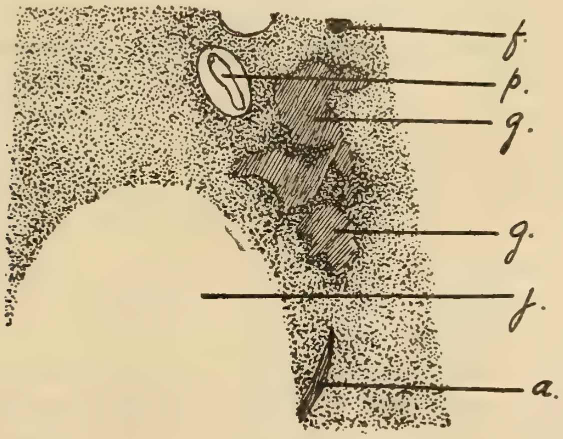

FIG. 2.

f. facial nerve ; *p.* posterior semicircular canal ; *g.g.* ganglion ; *j.* jugular fossa ; *a.* auricular branch of vagus nerve.

In the next section (fig. 3) the ganglion is seen to be much reduced in size, and now appears as one piece which is quadrilateral in shape. The facial nerve shows rather more in this section as it is beginning to turn downwards.

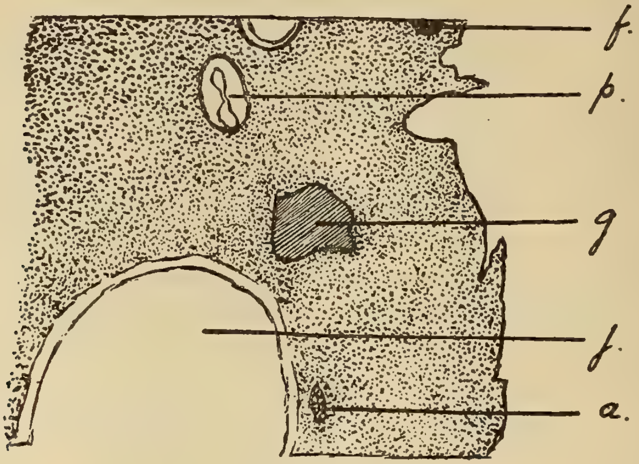

FIG. 3.

f. facial nerve ; *p.* posterior semicircular canal ; *g.* ganglion ; *j.* jugular fossa ; *a.* auricular branch of vagus nerve.

On coming to examine the minute structure of the ganglion there arises the difficulty that, in the human subject, the structures have undergone considerable *post-mortem* changes, owing to the fact that they cannot be put into the fixing fluid until a considerable time after death. Besides this difficulty another occurs in this special case, in that the fixing fluid takes some time to penetrate the bone which surrounds the ganglion. It is impossible, therefore, to give any satisfactory description of the minute intracellular appearances of the nerve-cells of the ganglion. As regards their general appearance, however, some points are obvious.

As a whole the ganglion contains a rather large proportion of nerve-fibres relative to the number of nerve-cells, Plate 6, fig. 4. The nerve-cells are present in numerous groups which are separated by bundles of fibres, and it is quite impossible to say whether all the fibres are connected with the nerve-cells of the ganglion or not.

As regards the cells themselves the majority are multipolar, and in this respect they differ from those found in the terminal ganglia of the eighth nerve, which are bipolar. In the stapedial ganglion there are also a few bipolar cells to be found in the upper portion (Plate 6, fig. 4), but this does not alter the general statement that the cells are multipolar in character.

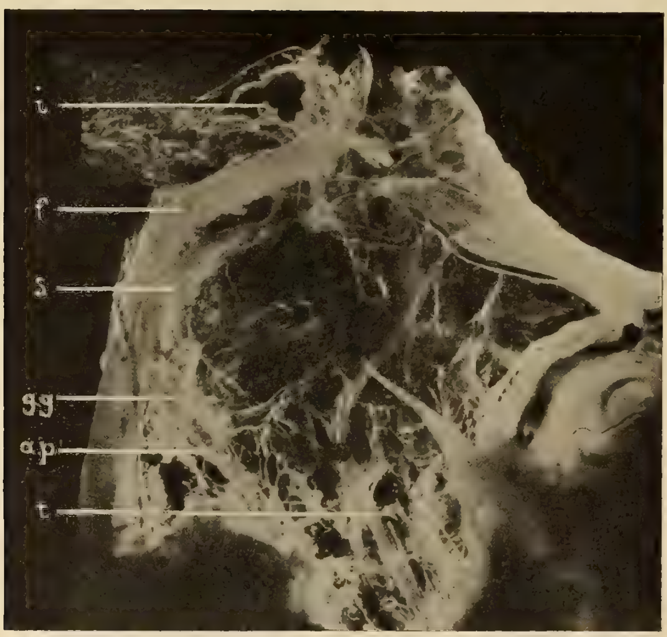

FIG. 1.

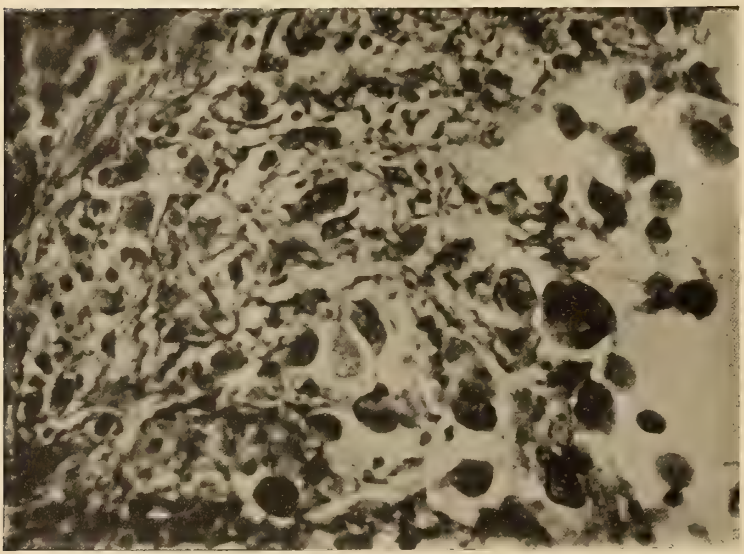

FIG. 4.

It is hardly possible at present to speak of the physiological significance of this ganglion. Experiment and careful pathological and clinical observation alone can determine this. There is, however, one very interesting clinical fact which is probably worth consideration in this respect. There is a very common form of deafness, unassociated with middle ear disease, and termed otosclerosis. Among other symptoms of this affection are, a diminution in the secretion of wax in the external meatus, and a diminished sensitiveness of the tympanic membrane and the posterior wall of the meatus. These are the regions supplied by Arnold's nerve which is formed by the union of bundles from the pneumogastric and facial nerves. As the stapedial ganglion is composed of fibres derived from both of these nerves it is very possible that autonomic fibres run from it to the parts mentioned. This would account for the diminished secretion of wax in the cases mentioned above.

DESCRIPTION OF PLATE 6.

FIG. 1.—The soft structure of the human middle ear.

i. incus ; *f.* facial nerve ; *s.* stapedius muscle ; *g.g.* ganglion ; *a.p.* auricular branch of vagus nerve ; *t.* nerve of Jacobson.

FIG. 4.—Section through ganglion showing the nerve cells. × 250 *ca.*

Studies on Enzyme Action. XIX.—*Urease: a Selective Enzyme.* II.—*Observations on Accelerative and Inhibitive Agents.*

By H. E. Armstrong, F.R.S., M. S. Benjamin and Edward Horton.

(Received January 1,—Read February 20, 1913.)

In the previous communication* experiments were described which had been made with the Urease present in the Soja bean proving that the enzyme is strictly selective in its action and that whilst its activity is much reduced by ammonia it is increased, in a remarkable manner, by the presence of carbonic acid: in other words, the two products of change affect the activity of the enzyme in opposite ways—a result altogether without precedent. In explanation of these results, the suggestion was made that Urease is a feebly acidic substance.

Though it was obvious that the results were not to be harmonised with the views that were current as to the manner in which enzymes act, we refrained from comment, deeming it desirable to obtain more information before discussing the new situation that was created. In the interval, the behaviour of other enzymes has been under observation by Dr. E. F. Armstrong and ourselves and it is proposed to discuss the general outcome of the work, in a comprehensive communication, at an early date. Meanwhile, we desire to bring forward an account of further observations on Urease carried out with the object of ascertaining the manner in which the activity of the enzyme is affected by the presence of various substances together with the urea.

Experimental Method.—In cases in which the substance to be added was easily soluble in water, solutions were prepared containing either one-half or one-tenth of a molecular proportion of the substance per litre. Having measured out 50 c.c. of a half-molecular solution of urea into each of two 200 c.c. Jena flasks fitted with indiarubber stoppers, 50 c.c. of water were added to the one and to the other 50 c.c. of the M/2 (or M/10) solution of the substance of which the effect was to be determined; each flask received also 25 c.c. of Soja extract (prepared as described in our former communication); all operations were carried out as near as possible at 25°.

As soon as the two flasks were charged, they were placed in an incubator which was maintained at 25°. After intervals of 5, 10, 15, 30, 45, 60, 75, 90 and 120 minutes, samples (10 c.c.) were withdrawn from each flask by means of pipettes previously warmed to 25°; each sample was run into a

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* "Studies on Enzyme Action. XV.—Urease: a Selective Enzyme," 'Roy. Soc. Proc.,' 1912, B, vol. 85, p. 109.

200 c.c. Erlenmeyer Jena flask containing a measured volume (an excess) of standardised chlorhydric acid. In all the experiments, the carbon dioxide present was removed by bubbling air through the mixture to which the standard acid had been added, after a few drops of olive oil had been introduced in order to prevent the frothing which otherwise occurs. At the end of an hour the excess of standard acid present was determined by titration with standard baryta solution, using litmus as indicator.

When dealing with substances of slight solubility (*e.g.* benzaldehyde, methylic salicylate, etc.), 105 c.c. of a solution were prepared having a concentration of M/4 as regards urea and M/20 as regards the substance to be added; 100 c.c. of the liquid taken out with a pipette were introduced into the 200 c.c. Jena flask and treated with 25 c.c. of Soja extract as before.

In most of the experiments made with the object of studying the action of carbon dioxide on more concentrated solutions of urea (semi-molecular, molecular, twice molecular and pentamolecular), in which samples were taken over a considerable period, the quantity of urea solution used was 200 c.c. together with 50 c.c. of Soja extract.

Influence of Acid Compounds on the Activity of Urease.

Not only strong acids but even the relatively much weaker carboxy-acids prevent the enzyme from acting, if present in appreciable amount. Thus no action took place in solutions of Aspartic and Salicylic acids of M/50 strength.

Boric Acid.—This acid has a remarkable depressant action when present in a solution containing the proportion $B_2O_3/50$ as shown in Table A (see Graph No. 12).*

Phenol.—In M/25 strength phenol itself has little influence but it appears to be sufficiently "acid" in M/5 strength to exercise a marked retarding effect (Table A, Graphs 1 and 2).

The influence of guaiacol and resorcinol is distinct from that of most other substances. At first these compounds retard the rate of hydrolysis but subsequently accelerate it slightly. Apparently, at the outset, they enter into competition with the enzyme and share the urea with it; as ammonia is liberated, however, they also serve to neutralise this base and therefore promote the change (Graphs 4, 5 and 6).

In the presence of quinol, action soon comes to an end (Table B). Only about 2 per cent. of change was effected when the solution was of M/25

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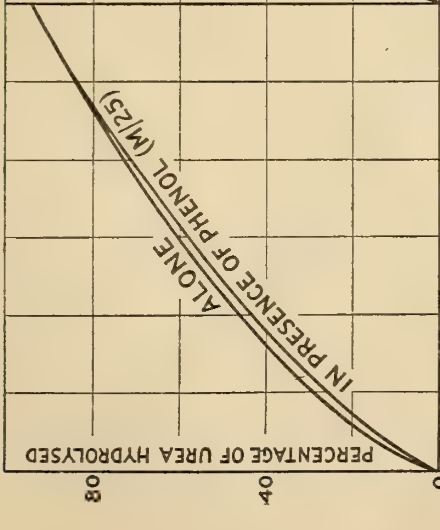
* Apparently boric acid is singular in that it retards the action of urease even in very weak solutions; all other acids, if present in sufficiently small amount, accelerate hydrolysis.

Table A.—Hydrolysis of Urea in M/5 Solutions containing Acidic Substances.

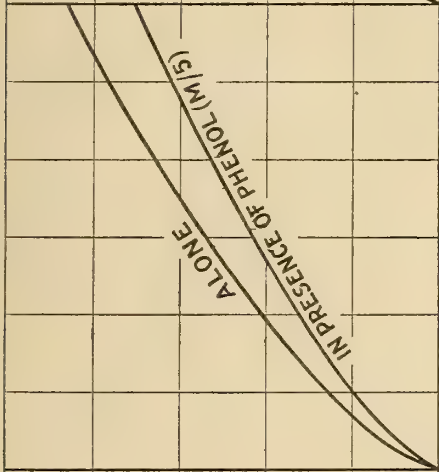
Time (mins.).	Percentage of urea hydrolysed.									
	Alone.	In the presence of phenol M/25.	Alone.	In the presence of phenol M/5.	Alone.	In the presence of resorcinol M/25.	Alone.	In the presence of resorcinol M/5.	Alone.	In the presence of guaiacol M/25.
5	10·8	9·2	9·9	7·1	10·6	9·4	9·5	6·5	11·8	9·2
10	17·7	15·5	15·8	11·9	17·7	16·2	15·3	12·6	17·1	15·3
15	23·8	21·2	20·6	15·9	23·3	22·4	20·8	17·6	22·7	20·6
30	37·4	35·6	33·9	26·5	36·7	37·7	32·9	30·9	36·0	34·8
45	49·5	47·8	44·9	35·3	49·2	50·6	43·2	42·6	47·4	46·2
60	60·8	58·4	54·2	43·4	59·1	61·2	52·5	53·3	56·7	56·9
75	69·7	68·8	63·5	50·7	67·6	71·4	60·5	63·2	65·8	66·4
90	78·2	78·2	71·5	57·7	76·5	81·0	68·3	72·1	74·2	75·1
120	94·4	94·4	86·0	71·2	90·9	95·6	81·6	89·9	88·2	90·1
	Alone.	In the presence of boric acid M/25.	Alone.	In the presence of glycine M/5.	Alone.	In the presence of glycine 2M/5.	Alone.	In the presence of glycine 4M/5.	Alone.	In the presence of asparagine M/5.
5	9·7	3·0	10·5	11·1	9·8	12·0	10·0	10·8	8·3	8·8
10	16·5	6·4	16·5	19·1	16·5	21·4	15·5	21·0	13·4	14·6
15	21·3	8·8	20·5	27·8	21·6	29·0	21·0	29·4	17·2	22·6
30	34·3	15·6	33·2	46·5	33·9	48·1	33·4	49·4	27·7	39·2
45	44·9	20·9	44·4	61·1	44·7	63·7	44·7	65·5	36·2	52·8
60	53·6	25·8	53·5	73·5	53·0	75·9	55·5	78·7	43·9	64·5
75	63·7	30·1	62·2	85·0	64·3	87·7	63·2	91·4	52·2	74·6
90	71·5	35·0	69·9	94·4	72·0	96·5	72·4	101·0	57·4	83·4
120	86·3	41·8	84·2	99·7	87·3	98·9	85·5	101·4	69·8	97·1

Table B.—Hydrolysis of Urea in M/5 Solutions containing Quinol and Monomethylquinol.

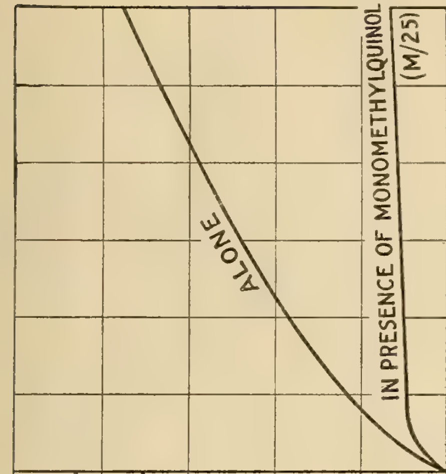
Time (minutes).	Percentage of urea hydrolysed.					
	Alone.	In presence of quinol M/500.	Alone.	In presence of quinol M/25.	Alone.	In presence of monomethylquinol M/25.
5	10·4	3·8	9·9	2·3	9·0	5·6
10	17·0	4·2	16·9	2·1	14·3	8·1
15	22·5	4·2	22·4	1·9	18·3	8·7
30	35·6	4·2	36·5	1·6	30·3	9·5
45	46·4	4·2	48·7	1·6	39·4	10·1
60	56·0	4·0	58·6	—	48·2	10·9
75	64·7	4·2	67·8	1·9	55·6	11·8
90	72·1	4·0	76·5	1·4	62·8	12·3
120	85·0	3·8	91·9	0·9	75·5	13·3



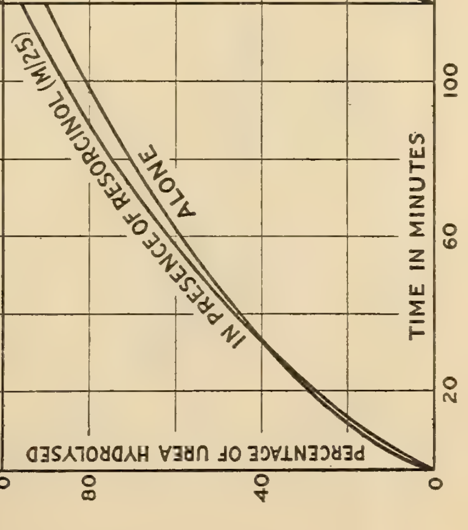
Graph 1.



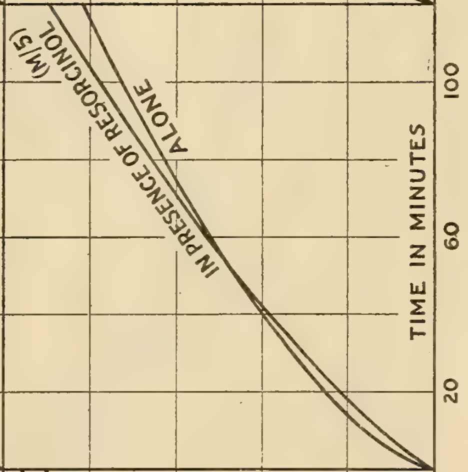
Graph 2.



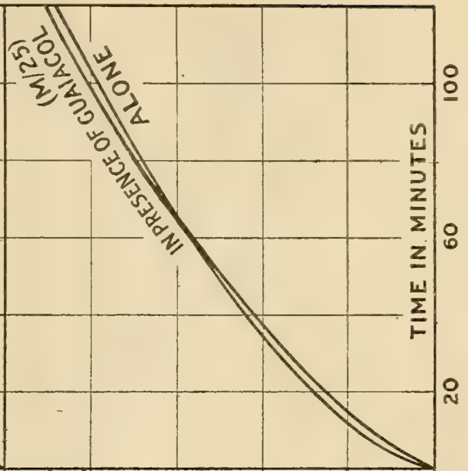
Graph 3.



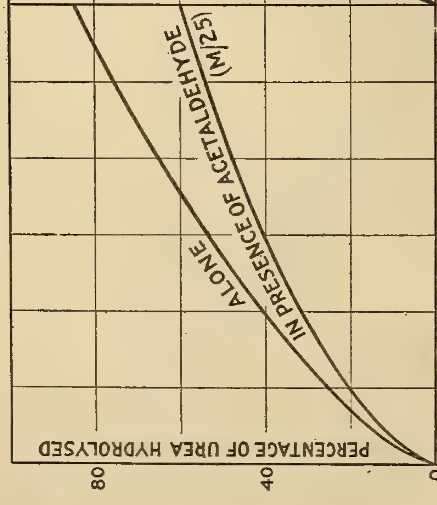
Graph 4.



Graph 5.



Graph 6.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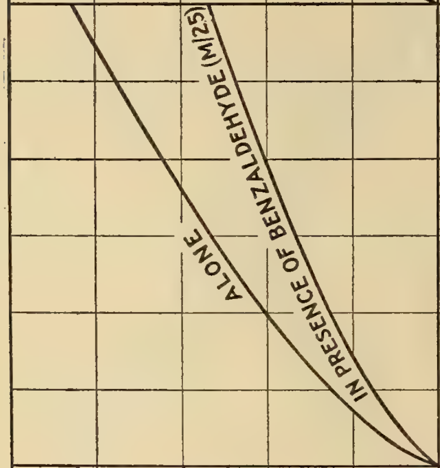
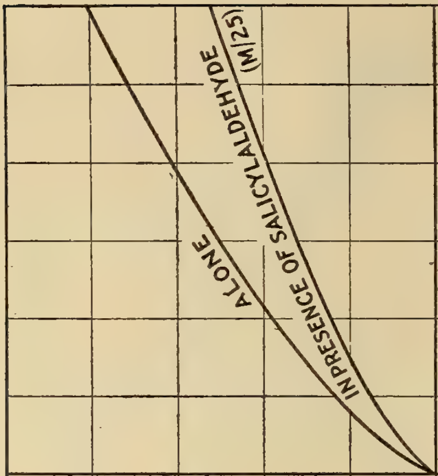
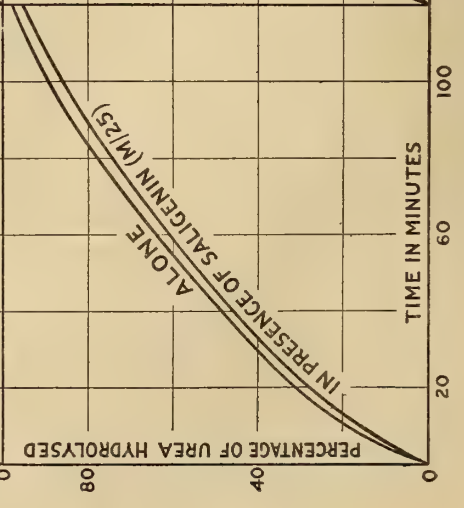
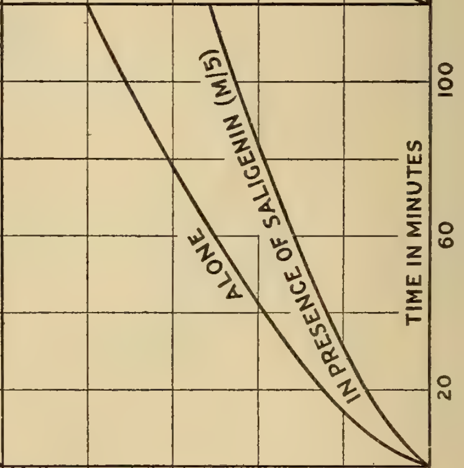
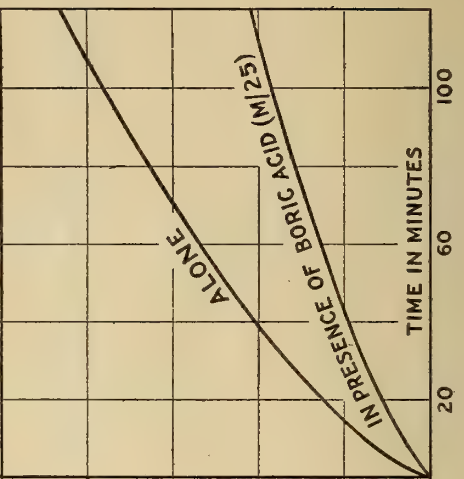

Graph 8.


Graph 9.


Graph 10.


Graph 11.


Graph 12.

strength and only about 4 per cent. when it was reduced to M/500; the solution rapidly darkened in colour. As no action takes place in presence of quinone (M/50), there can be little doubt that the effect produced by quinol is dependent on the production of this compound directly ammonia is present in sufficient amount to condition the oxidation of the quinol.

A series of experiments were made with the monomethylic-derivative of quinol, $C_6H_4(OH)\cdot OCH_3$, a compound of some interest, as it is formed together with quinol when arbutin is hydrolysed by emulsin.

The material used was that supplied by Kahlbaum. We were inclined at first to attribute its inhibitive power to the presence of quinol; we therefore purified it by distilling it *in vacuo* and made use of the intermediate fraction. The results given in Table B are those obtained with this product. We then digested the compound with ferric chloride, with the object of oxidising any quinol that might be present; after treatment with a little sulphite, to remove quinone, the residue was distilled *in vacuo*. As the substance thus purified was as active as the original material, we are inclined to think that in presence of ammonia and air monomethylated quinol is slowly converted into quinone and that this is the reason why it is so active an inhibitant (Graph 3).

Glycine and Asparagine.—These substances accelerate the rate of change as shown in Table A (Diagram 13).

Though they are "neutral" compounds, they neutralise both acids and bases; their marked accelerative effect is probably due to the fact that they serve to neutralise the ammonia as it is produced by the hydrolysis of the urea. As the positive influence of glycine is no greater apparently in more concentrated solutions, it is not improbable that it acts in two directions, both serving to fix ammonia and combining also to some extent with the enzyme.

Carbonic Acid.—A further series of observations carried out in presence of carbonic acid is given in Table C. The experiments were made in the manner already described (XV, p. 121). The results are represented by graphs in Diagram 15.

The four graphs in Diagram 14 are drawn from data given in XV, Part I. They represent comparable results obtained in experiments carried out simultaneously with the same sample of enzyme. It will be noticed that whilst the products of change taken together have but little influence, taken singly they are relatively very active but in opposite directions.

The set of graphs marked *c* (Diagram 15) show that when the proportion of urea is varied the difference observed in the absence of carbonic acid (XV, p. 117) is again apparent, the amount of change taking place in solutions

Diagram 13.

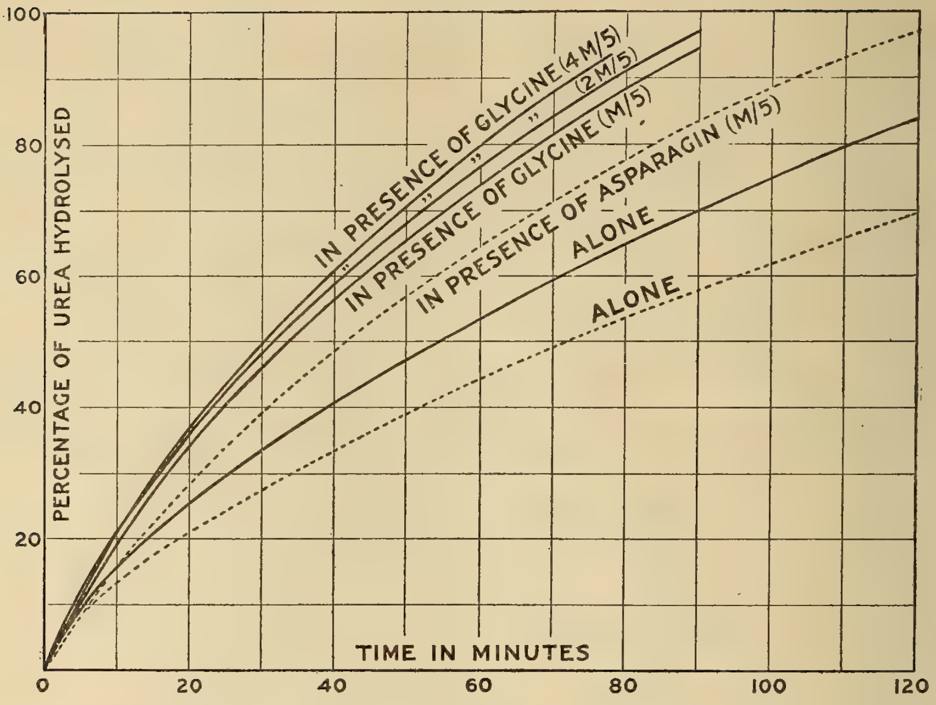


Diagram 14.

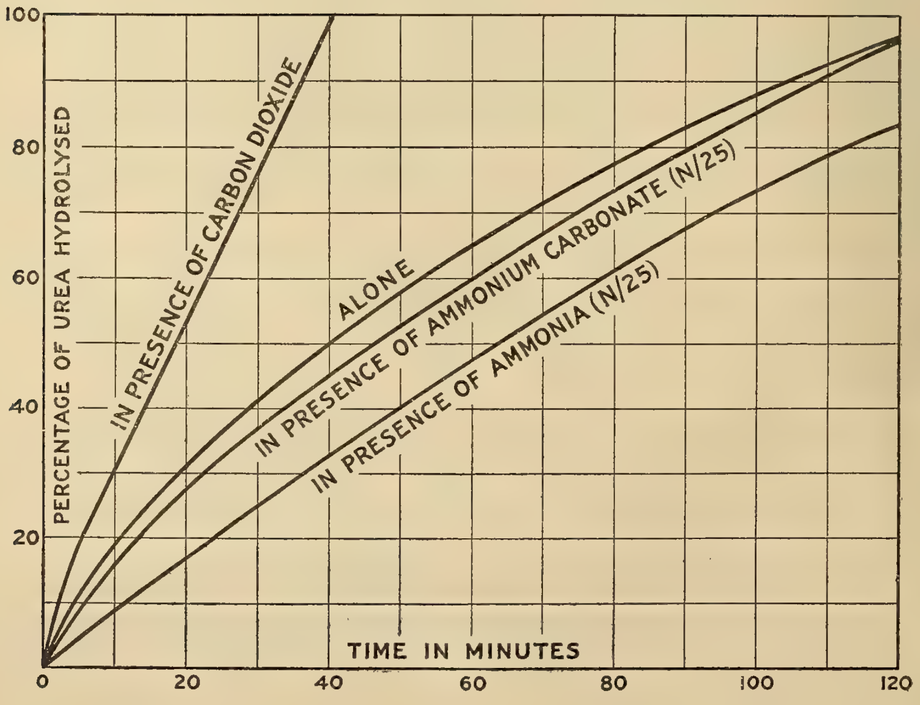


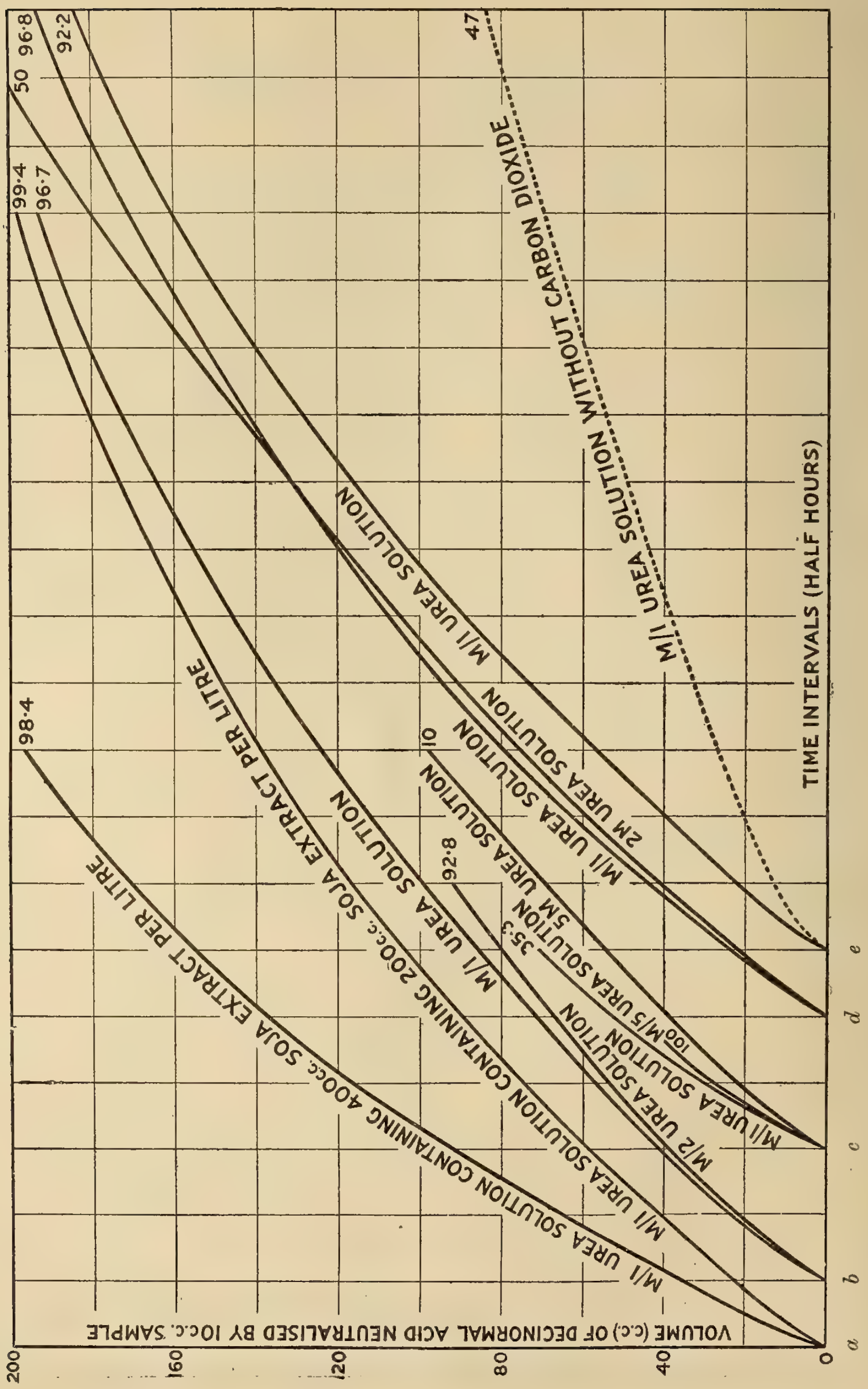
Table C.—Hydrolysis of Urea in Solutions saturated with Carbon Dioxide.

Time.	Volume of decinormal acid neutralised by 10 c.c. sample.										
	1. *Urea in M concentration.	2. Urea in M concentration.	3. Urea in M/5 concentration.	4. Urea in M concentration.	5. Urea in 5M concentration.	6. Urea in M/2 concentration.	7. Urea in M concentration.	8. Urea in M concentration.	9. Urea in 2M concentration.	10. Urea in M concentration.	11. †Urea in M concentration.
5 mins.	3·9	5·8	6·4	5·9	5·3	4·9	5·2	5·5	5·8	5·5	9·3
10	6·0	10·0	11·2	11·3	8·0	8·1	9·1	9·7	9·9	9·7	14·7
15	8·1	13·4	15·2	—	—	11·9	12·7	13·2	12·3	13·0	19·4
20	9·8	16·3	20·4	20·2	15·2	15·0	15·5	16·5	14·8	15·9	24·0
30	13·0	21·7	28·7	29·4	22·2	21·7	22·3	23·8	21·5	22·6	33·8
40	15·5	28·0	34·9	—	—	28·2	29·5	30·5	27·6	28·6	43·4
50	—	—	40·1	—	—	—	—	—	—	—	—
60	20·9	39·1	40·0	52·5	38·5	40·7	43·0	44·5	39·5	41·1	64·7
90	27·2	57·2		70·5	53·1	54·1	57·4	63·3	59·8	58·7	92·3
2 hours	33·2	73·7			—	66·4	70·6	80·0	76·7	75·2	116·1
2·5	—	—			—	79·8	—	—	—	—	—
3	44·3	103·8			100·3	92·8	99·2	107·8	104·7	103·1	155·2
3·5	—	—				99·4	—	—	—	—	—
4	55·5	—				99·2	124·9	131·8	132·0	128·9	185·3
4·5	—	—				99·2	—	—	—	—	196·8
5	64·7	151·3				99·4	146·5	152·9	156·7	—	200·6
6	74·8	169·0					164·4	171·5	181·3	166·1	200·6
7	84·0	184·3					181·1	187·0	—	181·1	
7·5	—	—					187·4	—	—	—	
8	92·7	197·2					193·3	199·1	218·7	193·7	
8·5	—	—					197·6		—	198·9	
9	—	200·4							—		
10	108·9	200·2							249·2		
24	187·6								353·9		

* No carbonic acid was present in this experiment.

† Twice as much enzyme was used in this case.

Diagram 15.


VOLUME (c.c) OF DECINORMAL ACID NEUTRALISED BY 10c.c. SAMPLE
200
160
120
80
40
0
TIME INTERVALS (HALF HOURS)
a
b
c
d
e
50
96·8
92·2
99·4
96·7
98·4
47
10
92·8
35·3
M/1 UREA SOLUTION WITHOUT CARBON DIOXIDE
M/1 UREA SOLUTION
2M UREA SOLUTION
M/1 UREA SOLUTION
M/1 UREA SOLUTION
5M UREA SOLUTION
100M/5 UREA SOLUTION
M/1 UREA SOLUTION
M/2 UREA SOLUTION
SOJA EXTRACT PER LITRE
M/1 UREA SOLUTION CONTAINING 200c.c. SOJA EXTRACT PER LITRE
M/1 UREA SOLUTION CONTAINING 400c.c. SOJA EXTRACT PER LITRE

of M/5 and M strength being almost the same, less change taking place in a solution of 5M strength. When change was complete in the weakest solution, only 1/5 of the urea in the solution of intermediate strength was hydrolysed and about 1/23 of that in the strongest solution.

To ascertain the optimum strength of solution, a comparative experiment was made with solutions of M/2 and M strength (the two graphs marked *b*). Again, the solution of molecular strength was found to be slightly the more active.

On contrasting the behaviour of solutions of molecular and twice molecular strength (the two graphs marked *d*), it was found that the change took place at very nearly the same rate in each, being slightly more rapid in the weaker during more than half the period of change.

Two experiments were made with solutions of molecular strength, twice the usual amount of enzyme being added to the one (the two graphs marked *a*); these gave results showing that the use of the larger proportion of enzyme is attended with a slight advantage.

The striking fact brought out in all the graphs representing experiments made in presence of carbonic acid is the approximation of the rate of change to a "linear" character.

To secure a more rigid comparison, smooth curves were drawn carefully to a large scale from the data obtained in the experiments and the rates of change were deduced by finding the value of the tangent at each of a series of points. The results are given in Table D.

It will be noticed that the influence of the acid increases as the action proceeds and that the rates are not far from being constant over considerable intervals. The values of the ratio $\frac{dx/dt}{a-x}$ in no way correspond to those to be expected in the case of a change proceeding at unimolecular rate, which is commonly regarded as the rate to which such actions tend to approximate.

Hydrogen Cyanide.—In view of the fact that hydrogen cyanide is a product of the hydrolysis of a considerable number of glucosides by "emulsin" and other enzymes, as well as on account of its remarkable physiological activity, it appeared to us to be important to study its behaviour towards an enzyme with which, presumably, it is not ordinarily brought into relationship. Our anticipation that it would act merely as a very weak acid and accelerate hydrolysis was proved to be correct. The results of a series of experiments with various strengths of the cyanide are given in Table E and as graphs in Diagram 16.

It will be seen that the accelerative influence increases with the

Table D.—Rates of Hydrolysis of Urea and Velocity Coefficients.

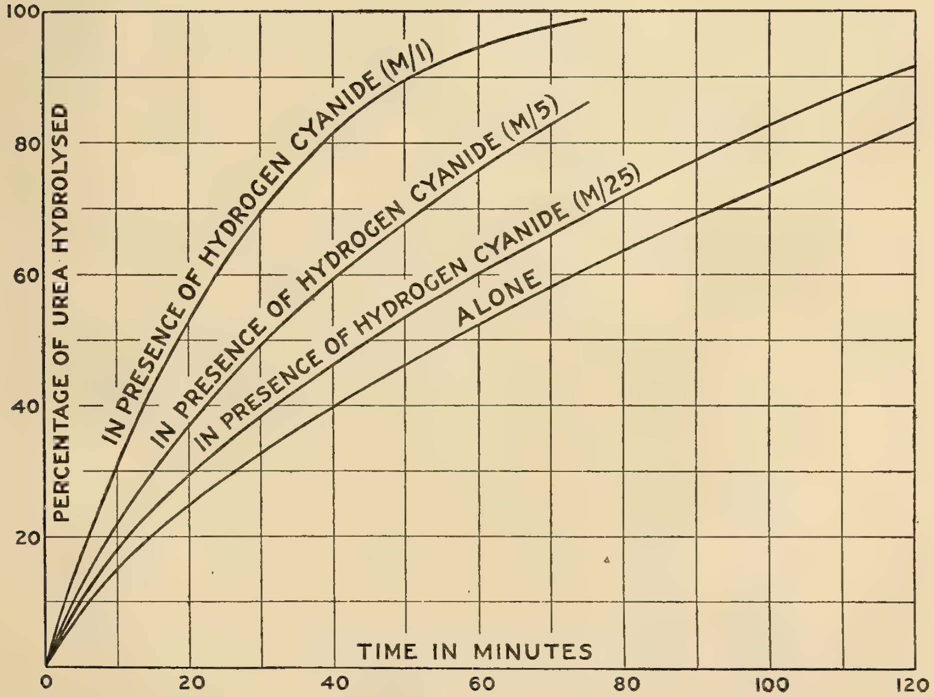
Grammes of urea hydrolysed (x).	Grammes of urea hydrolysed per hour (dx/dt)		Velocity coefficient, $\frac{dx/dt}{a-x}$	
	M Urea solution (aqueous).	M Urea solution (saturated with carbon dioxide).	M Urea solution (aqueous).	M Urea solution (saturated with carbon dioxide).
0·6365	1·623	3·583	0·113	0·250
1·273	1·171	2·616	0·085	0·191
2·546	0·872	2·558	0·070	0·205
3·819	0·795	2·527	0·071	0·226
5·092	0·732	2·489	0·074	0·252
6·365	0·668	2·329	0·078	0·270
7·638	0·605	2·113	0·082	0·288
8·911	0·560	1·948	0·092	0·320
10·184	0·515	1·687	1·073	0·351
11·457	0·458	1·445	1·297	0·409
12·730	0·401	1·216	1·775	0·539
14·003	0·337	0·981	3·420	1·058

Grammes of urea hydrolysed (x)	Grammes of urea hydrolysed per hour (dx/dt)		Velocity coefficient, $\frac{dx/dt}{a-x}$	
	M Urea solution saturated with carbon dioxide.		M Urea solution saturated with carbon dioxide.	
	Containing 200 c.c. enzyme per litre.	Containing 400 c.c. enzyme per litre.	Containing 200 c.c. enzyme per litre.	Containing 400 c.c. enzyme per litre.
1·591	2·851	4·436	0·212	0·330
3·182	2·730	4·379	0·231	0·370
4·774	2·470	4·354	0·241	0·425
6·365	2·215	4·086	0·256	0·473
7·956	1·973	3·647	0·280	0·517
9·547	1·706	3·112	0·312	0·572
11·139	1·426	2·762	0·368	0·713
12·730	1·177	2·291	0·513	1·004
14·321	0·910	1·801	1·318	2·601

Table E.—Hydrolysis of Urea in M/5 Solutions in presence of Hydrogen Cyanide.

Time (minutes).	Percentage of urea hydrolysed.				
	Alone.	In presence of hydrogen cyanide M/25.	In presence of hydrogen cyanide M/5.	Alone.	In presence of hydrogen cyanide, M.
5	8·8	10·9	12·6	9·7	17·0
10	15·6	18·5	21·9	16·5	31·4
15	20·4	24·0	—	21·3	42·2
20	—	—	36·3	—	53·3
30	32·9	38·6	49·6	34·3	69·1
40	—	—	—	—	81·5
45	43·4	49·8	63·1	44·9	—
50	—	—	—	—	89·7
60	52·2	59·7	75·4	53·6	94·3
75	61·4	69·2	86·2	63·7	98·8
90	68·7	77·6	94·8	71·5	—
120	83·1	91·6	100·0	86·3	—

Diagram 16.



concentration and becomes very considerable in solutions of molecular strength, 50 per cent. of the urea in such a solution being hydrolysed after an interval of about 18 minutes, whilst in the absence of the cyanide this amount is changed only after 55 minutes. In this case it was noted that the solution became brown.

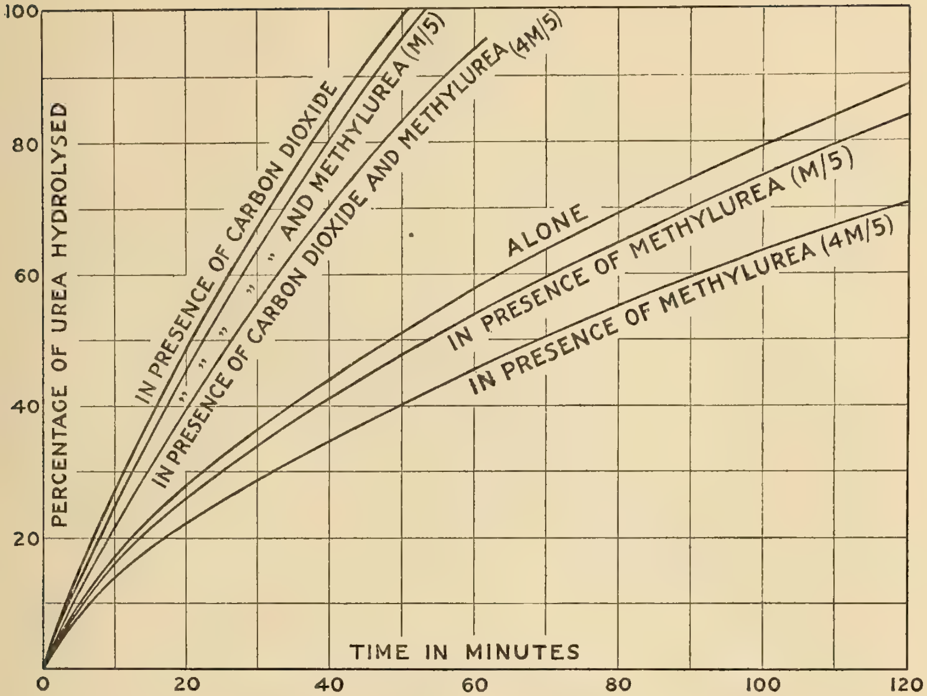
It is noteworthy that hydrogen cyanide has relatively less influence than carbonic acid in the later stages of the change.

Influence of Methylurea.—In Part XV it is shown that methylurea has a definite retarding effect. We have therefore carried out a further series of experiments in which, in one case, only the amount of methylurea present was varied, whilst in the other the action took place in presence of carbonic acid. The results obtained with this substance when used in presence of carbonic acid are of special interest in view of the close relationship of urea and methylurea; they are given in Table F and in Diagram 17. It will be noticed that the addition to the M/5 solution of urea of an equivalent amount of methylurea has a marked depressant effect and that when the urea and the methylurea are present in the ratio 1 : 4 the effect of the neutral substance is considerable. As practically the same alteration in osmotic conditions would be produced by equivalent proportions of urea and methylurea, it is to be supposed that the influence exercised by methylurea is due in part to the fact that it shares the acid enzyme with the urea but it also interferes mechanically. The results obtained in presence of carbonic acid are similar to those obtained in its absence but action proceeds at accelerated rates.

Table F.—Hydrolysis of Urea in M/5 Solutions in presence of Methylurea.

Time (minutes).	Percentage of urea hydrolysed.					
	Alone.	In presence of methylurea M/5.	In presence of methylurea 4M/5.	In presence of carbon dioxide.	In presence of methylurea (M/5) and carbon dioxide.	In presence of methylurea (4M/5) and carbon dioxide.
5	10 ·0	9 ·7	8 ·3	15 ·9	15 ·3	11 ·7
10	16 ·7	15 ·9	13 ·4	27 ·8	25 ·0	22 ·0
15	22 ·9	21 ·4	18 ·0	36 ·9	35 ·4	30 ·9
20	—	—	—	49 ·0	46 ·0	39 ·6
30	36 ·0	34 ·3	28 ·4	66 ·6	62 ·8	55 ·1
40	—	—	—	83 ·9	80 ·6	70 ·8
45	47 ·9	44 ·1	37 ·3	—	—	—
60	57 ·0	53 ·4	45 ·2	97 ·9	98 ·6	94 ·1
75	66 ·8	62 ·1	53 ·0	—	—	—
90	74 ·0	70 ·0	59 ·6	99 ·6	99 ·8	99 ·6
120	88 ·4	83 ·9	70 ·6	—	—	—

Diagram 17.



Influence of Neutral Agents which depress the Activity of Urease.

Alcohols.—Ethylic and propylic alcohols exercise moderate effects which may be attributed to the changes they produce in the osmotic conditions. As in all other cases studied, the less soluble alcohol is the more active (Diagram 18).

Saligenin, $C_6H_4(OH) \cdot CH_2(OH)$, is far more active than either of the paraffinoid alcohols (Graphs 10 and 11).

Aldehydes.—In the presence of formaldehyde (M/25), action comes to an end when about 4 per cent. of change has taken place.

Acetic aldehyde, benzoic aldehyde and salicylic aldehyde are moderately active depressants; the results obtained with these substances and with saligenin are given in Table G and in Graphs 7, 8 and 9.

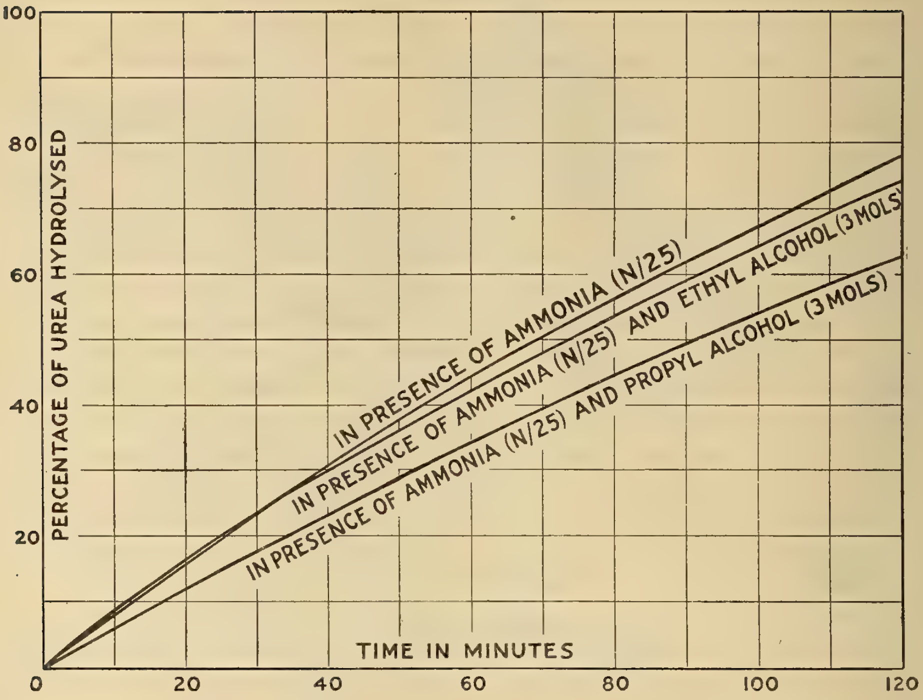
The observation that glucose has a slight retarding effect has been confirmed.

Sodium salicylate and methylic salicylate have practically no action.

We are inclined to think that the aldehydes are all, in some measure, chemically active towards urease and that even saligenin may be credited with slight chemical activity.

It has been customary to regard the action of enzymes as subject to the

Diagram 18.



"law of mass action" and to assume that the rate at which action takes place is such that it is proportional at any moment to the amount of substance left unchanged. It should be possible, therefore, to express the rate of such changes by logarithmic curves but it is recognised that the products of change have a more or less marked retarding influence and that, on this account, the actual curve expressing the rate of change always falls below the theoretical curve. It is further supposed that the action is reversible and that therefore, on this account, the change is never complete, though it may be very nearly so in dilute solutions. Lastly, it is recognised that when the enzyme is present in very small proportion, the action proceeds at a nearly constant rate: also that it is much retarded in very concentrated solutions—a result ascribed by some to the viscosity of such solutions.

It appears to us that our results are not in accord with the views hitherto accepted and that it is to be supposed that enzymic changes would be found to take place at approximately constant rates were it not that they are subject directly and indirectly to considerable retardation by the products of change; indeed it is probable that the products of change have an affinity for the

Table G.—Hydrolysis of Urea in M/5 Solutions containing Alcohols and Aldehydes.

Time (mins.).	Percentage of urea hydrolysed.							
	Alone.	Plus ethylic alcohol 1 mol.	Plus propylic alcohol 1 mol.	Alone.	Plus ethylic alcohol 3 mols.	Plus propylic alcohol 3 mols.	Alone.	In presence of saligenin M/25.
5	2 ·7	2 ·9	2 ·8	4 ·9	3 ·7	2 ·5	11 ·7	9 ·8
10	7 ·0	6 ·9	6 ·6	7 ·9	8 ·7	6 ·5	19 ·9	16 ·5
15	11 ·2	11 ·4	10 ·9	12 ·4	12 ·2	8 ·8	26 ·1	23 ·7
30	22 ·5	21 ·5	21 ·0	23 ·2	23 ·5	17 ·9	40 ·3	37 ·4
45	32 ·6	31 ·8	30 ·8	34 ·8	33 ·1	26 ·2	—	—
60	42 ·0	41 ·5	40 ·5	44 ·6	42 ·1	34 ·5	64 ·3	—
75	51 ·2	50 ·4	49 ·0	53 ·3	50 ·9	41 ·9	74 ·4	—
90	60 ·2	57 ·0	57 ·3	61 ·8	58 ·9	49 ·6	84 ·0	80 ·6
120	75 ·2	73 ·3	73 ·0	78 ·1	74 ·3	62 ·7	97 ·4	95 ·5
12 hrs.	98 ·1	98 ·7	98 ·7	—	—	—		
	Alone.	In presence of saligenin, M/5.	Alone.	In presence of acetic aldehyde, M/25.	Alone.	In presence of benzoic aldehyde, M/25.	Alone.	In presence of salicylic aldehyde, M/25.
5	10 ·4	4 ·7	10 ·6	8 ·5	9 ·4	5 ·4	8 ·5	5 ·6
10	16 ·7	9 ·2	16 ·1	13 ·1	16 ·0	8 ·9	14 ·6	9 ·4
15	20 ·5	12 ·2	21 ·0	17 ·2	20 ·5	12 ·5	19 ·3	12 ·0
30	32 ·5	19 ·3	33 ·5	26 ·1	33 ·7	21 ·2	31 ·1	20 ·0
45	41 ·5	26 ·1	43 ·9	34 ·3	44 ·3	27 ·8	41 ·9	26 ·8
60	50 ·9	31 ·5	53 ·6	40 ·5	53 ·9	33 ·7	50 ·9	32 ·0
75	60 ·3	37 ·7	63 ·4	46 ·4	62 ·9	39 ·3	59 ·3	38 ·1
90	66 ·9	42 ·6	70 ·8	51 ·3	70 ·4	44 ·0	66 ·6	43 ·1
120	80 ·4	51 ·8	84 ·8	60 ·2	86 ·0	53 ·7	81 ·0	52 ·5

In the experiments with ethylic and propylic alcohols in weight normal solutions, an amount of ammonia equivalent to one-tenth of that ultimately produced was added initially to the urea solutions.

enzyme which is actually greater than that which obtains between the hydrolyte and the enzyme.

It has often been suggested that the enzymes are colloids. The experiments carried out in the course of this series of studies appear to justify the belief that enzymic action takes place entirely at the surfaces of colloid particles suspended in the solution of the hydrolyte and not between substances which are all in true solution.

The subject will be more fully discussed in the later communication to which we have referred.

[The cost of this investigation has been partially met by a grant for which I am indebted to the Government Grant Fund of the Royal Society.—H. E. A.]

A Preliminary Note on the Fossil Plants of the Mount Potts Beds, New Zealand, Collected by Mr. D. G. Lillie, Biologist to Captain Scott's Antarctic Expedition in the "Terra Nova."

By E. A. Newell Arber, M.A., Sc.D., F.G.S., F.L.S., Trinity College, Cambridge, University Demonstrator in Palæobotany.

(Communicated by Prof. T. McKenny Hughes, F.R.S. Received February 17,—Read March 6, 1913.)

[Plates 7 and 8.]

In the present communication, I propose to discuss very briefly the first fruits, which have reached this country, of Captain Scott's Second Antarctic Expedition (1910–13). A full account of the fossil flora in question must be reserved for a future occasion. At present I have only permission to contribute a preliminary note on the subject.

It is well known that, during the winter months of the last two years, the "Terra Nova," the ship of Captain Scott's Second Antarctic Expedition, has been actively engaged in furthering scientific researches in New Zealand waters, returning, however, to the Antarctic each summer. My friend, Mr. D. G. Lillie, B.A., of St. John's College, Cambridge, one of the biologists of Captain Scott's Scientific Staff, who has been attached throughout to the "Terra Nova," has been busily occupied with various researches, partly biological and partly geological. During the short periods when he has been free to proceed with geological work, he has set himself the task of trying to clear up some of the doubtful points, which remain unsolved, in regard to the stratigraphical geology of New Zealand, more especially by means of the fossil floras of the rocks in question. As is well known, the precise geological age of many subdivisions of the stratigraphical sequence of these islands remains in doubt, and in some cases these questions have been matters of keen dispute in the past as at the present time. Among them, none has given rise to greater controversy than the doubt which has existed as to the precise geological age of the plant beds of Mount Potts, in Ashburton County, Canterbury. Do these beds contain *Glossopteris,* and perhaps a typical Permo-Carboniferous Glossopteris flora? Did New Zealand, as one would expect, in Permo-Carboniferous times form part of the great Southern continent, "Gondwanaland," the home of the Glossopteris flora, like the greater part of Australia, South Africa, and South America? These are the questions as yet in doubt. If, on the other hand, New Zealand, in Permo-Carboniferous times, formed no part of Gondwanaland, we are obviously face to face with a conclusion of the greatest geological importance. This is one of the questions which Mr. Lillie has set himself the task of solving.*

Plant-remains in the Mount Potts beds were first discovered by A. McKay† in 1878. The collection was examined by Hector‡ in the same year, and he stated that it contained examples of *Glossopteris* and *Schizoneura,* this assertion being repeated in 1886. These conclusions subsequently led to considerable controversy between Hector, Haast, McCoy, and others, the details of which it is unnecessary to enter into here. The whole question has turned on the identification of the fossils, and the evidence as to their stratigraphical position. Further, until Mr. Lillie's visit, the collections from this region appear to have been small and very fragmentary, and even these had not been examined at any time by European specialists in palæobotany.

The fine collections made, in November, 1911, by Mr. Lillie, in conjunction with Mr. R. Speight of Canterbury College, in very wild and difficult country, appear, however, to settle this question once and for all. *Glossopteris* itself is not present, nor is the flora a typical Glossopteris flora.

The most characteristic and striking plant represented is, however, one which resembles *Glossopteris* in habit. It has the same tongue-shaped, entire frond, with a well-marked midrib, but the lateral nerves, instead of anastomosing as in *Glossopteris,* do not unite with one another.§ (Plate 7, figs. 1 and 4.) One member of this genus has already been described from the Rhætic beds of Chili. The specimens in question were termed *Lesleya Steinmanni.*‖ It seems to me very doubtful whether these leaves belong to the Palæozoic genus *Lesleya.* I should be inclined to place the New Zealand fossils, at any rate, in a new genus *Linguifolium*, and to regard them as a new species *L. Lillieanum*, so named in honour of Mr. Lillie. They are certainly specifically distinct from the Chilean plant. This plant may be also compared with the *Copiapaea plicatella* of Solms* from Chili, and the *Blechnoxylon talbragarense* of Etheridge† from New South Wales, the latter believed to be a Palæozoic fossil.

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* This paper was written before the news of the sad disaster to the Polar party of the Expedition reached this country. It is, however, only fair to the memory of the late Capt. Scott, whose death I deplore most sincerely, to point out that the work which Mr. Lillie and others have been engaged in, during the winter months in New Zealand, was part and parcel of the scientific intention of his expedition, to be fulfilled during the times when the "Terra Nova" would be useless in the Antarctic, but could be profitably employed in New Zealand waters.

† McKay, 'Rep. Geol. Explor. Geol. Surv. N.Z.,' 1878, pp. 91–109.

‡ Hector, 'Proc. N.Z. Inst.,' 1878, vol. 10, p. 533, and 'Cat. N.Z. Court, Indian and Colonial Exhibition, London,' 1886, p. 77.

§ As will be seen from the upper part of the photograph on Plate 7, fig. 1, there appear to be at least some anastomoses, but I am convinced that, in this as in other cases, these are false and not real, and that they are due either to the partial removal of the film of carbon, or to the fact that the normal distribution of the nervation had become disturbed just before or during preservation. Such false anastomoses are by no means infrequently met with among fossil impressions.

‖ Solms, 'Neues Jahrb.,' 1899, vol. 12, Beil. Bd., p. 596, Plate XIII, figs. 5–7.

In addition to *Linguifolium Lillieanum*, a number of other well preserved species occur. There is a new species of *Chiropteris* (Plate 8, fig. 6), the distal margin of which is lacerated, and which I propose to name *C. lacerata sp. nova.* Leaves of a species of *Baiera* (Plate 7, figs. 1, 2, and 3), closely similar to but perhaps distinct from *Baiera paucipartita*, Nathorst, from the Rhætic of Bjuf, Sweden, are also associated. A *Dictyophyllum*, which may be closely compared with *Dictyophyllum acutilobum* (Braun), is present. Other fronds are those of *Thinnfeldia lancifolia* (Morris) (Plate 8, fig. 7) and *Cladophlebis australis* (Morris). Numerous examples of a *Tæniopteris*, which is no doubt *T. Daintreei*, McCoy, occur (Plate 8, fig. 5). Among the Equisetaceous remains, pith-casts are represented which resemble those of *Phyllotheca* or *Schizoneura*, but, in the absence of foliage, it is impossible to refer them to the one genus rather than the other. However, the small detached leaf-sheaths of a *Phyllotheca* are undoubtedly present. Finally, associated with the above plants, are many examples of the Indian (Gondwana) *Palissya conferta* (Oldh.) (Plate 8, fig. 5).

From this brief review of this interesting flora it is obvious that it is of Mesozoic age, and belongs either to the late Triassic (Rhætic) or to the early Jurassic period. *Linguifolium*, which is a homœomorph of *Glossopteris*, just as *Lonchopteris* is of *Alethopteris*, or *Dictyozamites* of *Zamites*, is already known from Rhætic rocks in Chili. *Chiropteris* is at present confined to the Rhætic, some species occurring in the Triassic rocks of Europe, and also, as Prof. Seward has shown, in the Stormberg Series (Rhætic) of South Africa. *Baiera paucipartita*, Nath., and *Dictyophyllum acutilobum* (Braun) occur in the Rhætic of Europe. *Thinnfeldia lancifolia* (Morris) is found chiefly in the Rhætic, though it no doubt also occurs in the Jurassic. *Cladophlebis australis* (Morris) is known both from the Jurassic and Rhætic in the Southern Hemisphere.

The only two plants which, at present, are exclusively Jurassic are the Gondwana (Rajmahal) *Palissya conferta*‡ (Oldh.), and *Tæniopteris Daintreei*,

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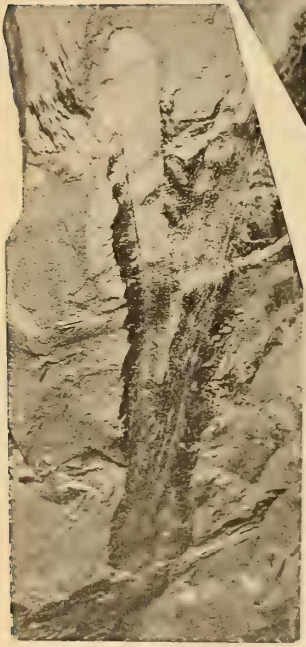
* Solms, *ibid.*, 1899, p. 594, Plate XIII, figs. 8–11.

† Etheridge, 'Rec. Austral. Mus.,' 1899, vol. 3, p. 135, Plates XXIV–XXVII.

‡ It is interesting to find that Dr. Halle has just described this plant from Graham Land in the Antarctic. ('Wissen. Ergebn. Schwed. Südpolar-Exped., 1901–1903,' 1913, vol. 3, Part 14, p. 86, Plate VIII, figs. 26–40.)



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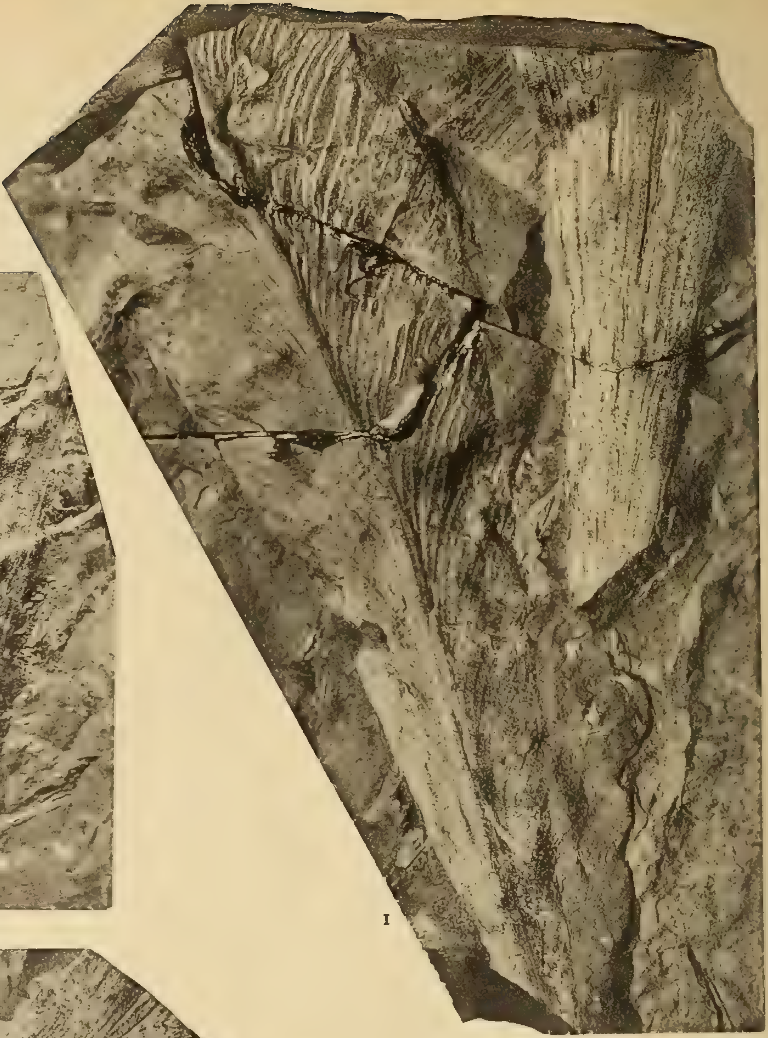

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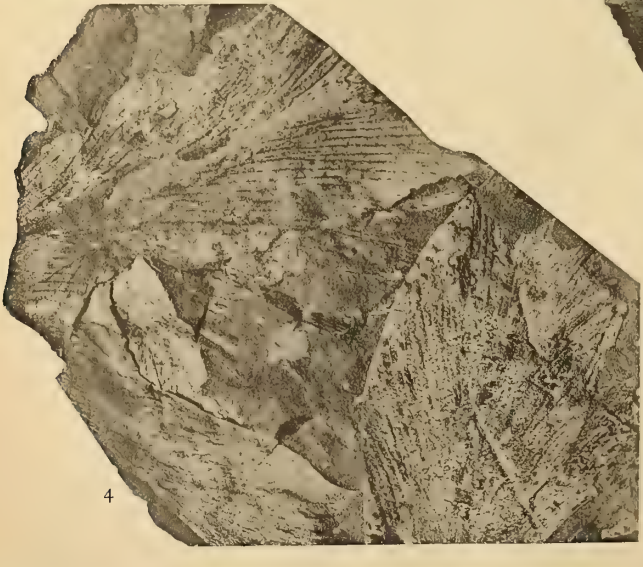

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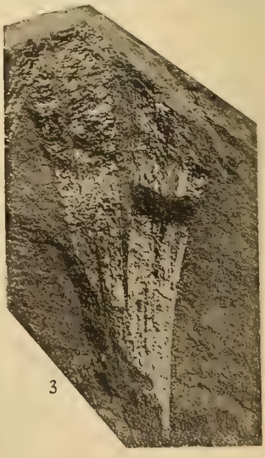
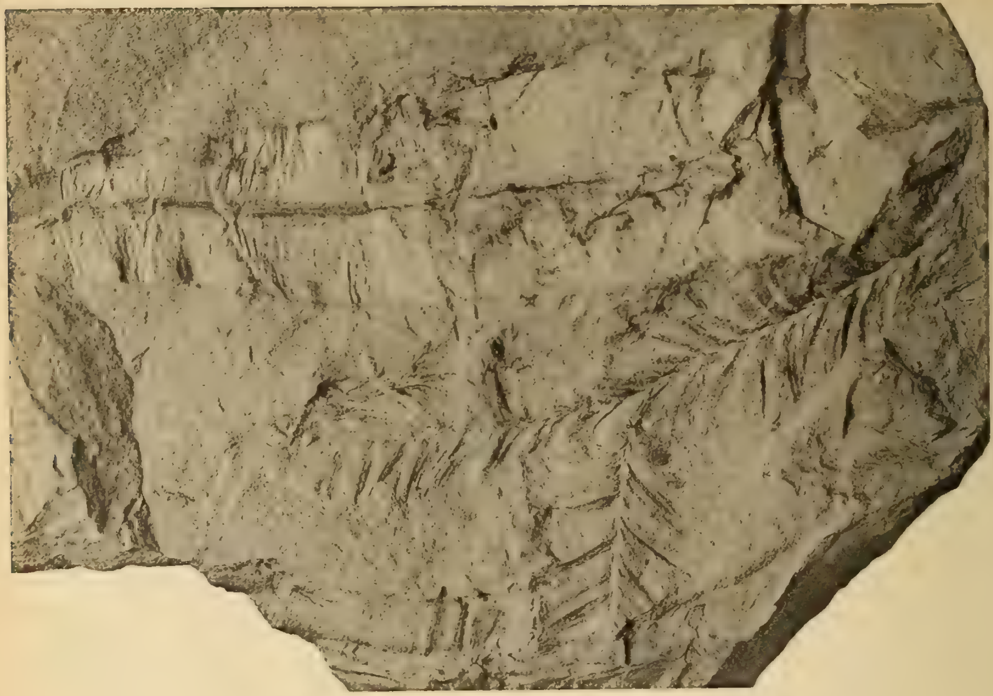


3

Photos by W. Tams.

FOSSIL PLANTS FROM NEW ZEALAND.



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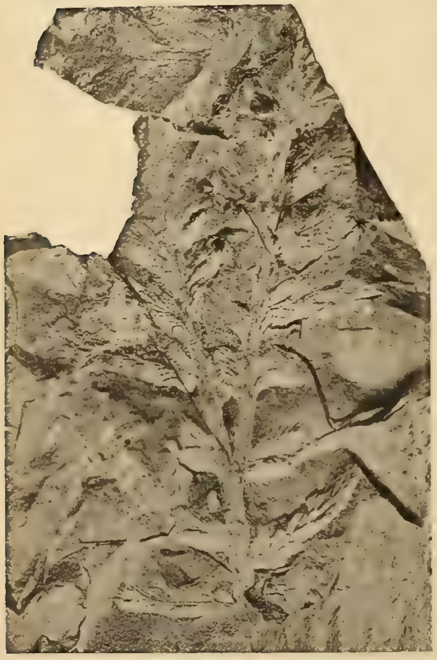
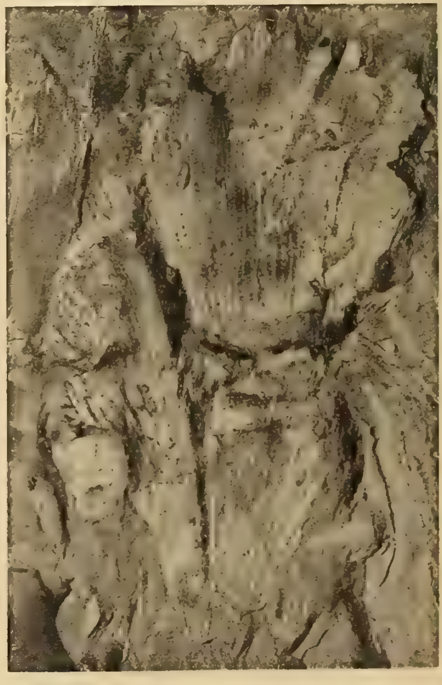


7



6

Photos by W. Tams.

FOSSIL PLANTS FROM NEW ZEALAND.

McCoy, which, in Australia, is essentially a Jurassic type, though perhaps it may also occur in the Rhætic.

From this rapid survey of the Mount Potts flora we see that, while its affinities are essentially Rhætic, a few Jurassic types also occur, and thus the age of the beds may be either Rhætic or Lower Jurassic. At present we are unable to distinguish clearly between a Rhætic and a Lower Oolite flora, so this point need not be laboured here.

There is little doubt that the Mount Potts beds are, geologically, the oldest, plant-bearing series as yet discovered in New Zealand, and as we have seen they are of Rhæto-Jurassic age. Palæozoic sediments with marine invertebrates undoubtedly occur in the islands, but so far there is no evidence of any land floras of older age than the Rhætic. Not only is *Glossopteris* unknown from New Zealand, but no land plants of Palæozoic age of any description have ever been found there. There is thus no evidence that New Zealand ever formed part of Gondwanaland, and this is a conclusion of great theoretical interest.

EXPLANATION OF THE PLATES.

(All the photographs are by Mr. W. Tams, Cambridge. Nearly all the figures are enlarged.

Plate 7.

Fig. 1.—*Linguifolium Lillieanum gen. et spec. nova.* On the left, an almost entire leaf, the apex being wanting however. On the right *Baiera sp.*, the base of a leaf. Enlarged $\times \frac{3}{2}$.

Fig. 2.—*Baiera cf. Baiera paucipartita* Nath. An almost complete leaf. Enlarged $\times \frac{3}{2}$.

Fig. 3.—*Baiera cf. Baiera paucipartita* Nath. A median portion of a leaf. Nat. size.

Fig. 4.—*Linguifolium Lillieanum gen. et spec. nova.* Two fragments of leaves, one nearly apical, the other median, showing the nervation clearly. Enlarged $\times \frac{5}{3}$.

Plate 8.

Fig. 5.—*Tæniopteris Daintreei* (McCoy) above, and *Palissya conferta* (Oldh.) below. Enlarged $\times \frac{3}{2}$.

Fig. 6.—*Chiropteris lacerata sp. nova.* A nearly complete leaf, showing the incised apex. Enlarged $\times \frac{3}{2}$.

Fig. 7.—*Thinnfeldia lancifolia* (Morris). Nat. size.

On the Nature of the Toxic Action of Electric Discharge upon Bacillus coli communis.

By J. H. Priestley and R. C. Knight.

(Communicated by J. Bretland Farmer, F.R.S. Received February 13,—Read April 10, 1913.)

Introduction.

In a recent paper, Thornton* has drawn attention to some results he had obtained in experiments upon the bactericidal action of electric discharge. Plates of agar were infected with bacteria of various species, and subjected, under different conditions, to the discharge from an electrified point. The plates of agar were subsequently incubated and observations taken of the development of colonies from the surviving bacteria. From experiments upon these lines he concluded that the ionised air, *i.e.* the small current (the whole of the current passing from the point was about 4 micro-ampères) produced by his discharge methods, proved fatal after longer or shorter periods to all the species of bacteria subjected to it.

This conclusion is of considerable interest, suggesting, as it does, the possibility of electrical treatment of tissue attacked by pathological bacteria, with a view to retarding bacterial action. Our attention was attracted to this paper by the fact that its conclusions seem at variance with some conclusions previously arrived at by one of us in conjunction with Miss E. M. Lee, in an investigation carried out at the University of Bristol, of which only a brief preliminary note has so far been published,† pending the further experiments which Miss Lee hopes to be able to carry out.

In this research cultures of the sour-milk bacillus, *B. Bulgaricus*, were subjected to small electric currents, and observations were made to determine the effect of such treatment upon their vitality. Contrary to Thornton's experience it was found that current densities below about 58 micro-ampères per square centimetre served to increase both the fermentation power of the bacteria as determined by electrical conductivity, and also the rate of growth as determined by countings. The fact that the current density required to produce any inhibitory effects in these experiments had to be greater than about 60 micro-ampères per square centimetre may have been due to the fact that in these cases the electric current was derived from a source of comparatively low voltage, and transmitted to the nutrient medium through the ordinary form of Kohlrausch platinum electrode which was immersed in it. But this suggestion immediately raises the question as to whether the effect detected by Thornton bore any relation to the direct action of the current, or was connected with the chemical changes produced in the atmosphere surrounding the discharge point. Thornton considers that the fatal result of the discharge may be wholly attributed to "the direct influence of, and contact with, ions in the electric wind." It is hardly conceivable, however, that mere ionic bombardment could be responsible for such deep-seated action as was observed, especially in consideration of the fact that ions have practically no penetrating power in the presence of water, a film of which must have always intervened between the organism and the discharge.

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* "Influence of Ionised Air on Bacteria," 'Roy. Soc. Proc.,' 1911, B, vol. 84, p. 280.

† "The Influence of Electricity on Micro-organisms," J. H. Priestley and E. M. Lee, 'Brit. Assoc. Report,' 1911, p. 603.

Foulerton and Kellas,* as the result of experiments carried out along lines similar to those described by Thornton, employing in many cases the same species of bacteria, had previously arrived at the conclusion that electric discharge itself was not deleterious to the organisms. They found that "emulsions" of bacteria in water became sterile after subjection to the discharge in air and in various artificial atmospheres, but considered that the fatal effect was due, not to the current, but to the products of the discharge, viz., nitric and nitrous acids in air and hydrogen peroxide in hydrogen. Qualitative and quantitative tests of distilled water, after subjection to the discharge, revealed the fact that these substances were indeed present in measurable quantities, and subsequent trials showed that such concentrations of them were fatal to bacteria, independent of the discharge. It is possible that the results obtained by Foulerton and Kellas cannot be directly applied to explain Thornton's experiments, because of the different electrical conditions. In their experiments the bacteria were contained in water in a test-tube and the current was discharged from the points of a platinum brush suspended over the surface, earth connection being made through a platinum wire sealed into the bottom of the tube. In all cases the high-tension discharge from the brush of platinum points was oscillatory in character, and it might therefore be expected that any effects produced by the action of the discharge upon the atmosphere would be enhanced, while effects due to direct action of an electric current should be far less apparent.

The results obtained by Thornton with the apparatus depicted in his fig. 2 suggest that the products of discharge, and not the ions, were the active factor. In these experiments the current passed, not through the bacteria-infected plate to earth, but through the air between two metal points above the culture, and in this case, where the current through the bacteria was a minimum, "the (sterilising) action was much stronger than in the first arrangement," in which one point discharged directly on to the culture. The investigation, of which an account is presented below, was therefore commenced with the intention of attempting to ascertain whether current densities of the order used by Thornton, obtained from a high-tension source, could still prove toxic when the influence of all toxic substances produced by the chemical action of the discharge had been eliminated.

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* "Action on Bacteria of Electrical Discharges," 'Roy. Soc. Proc.,' 1906, B, vol. 78, p. 60.

Experimental.

Bacillus coli communis, being found by Thornton to be one of the least sensitive organisms he employed, was chosen for the experiment. The high-tension discharge was obtained from the ordinary 100-volt direct-current circuit by leading this current after interruption by a mercury break through the primary of a large induction coil. The alternating discharge from the secondary was then obtained as a continuous positive and negative charge at either side of a spark gap by leading the alternating discharge through five Lodge valves arranged in series; these valves act as rectifiers, only permitting the current to pass in one direction owing to the structure of the electrodes. This apparatus, which was purchased from a special research grant obtained from the Board of Agriculture and Fisheries, was available during the intervals when not required for other experiments in progress in the Department. By this method it was then easy to maintain as long as required a difference of potential of some 70,000 volts between the poles of the spark gap, one pole was then connected to earth and the other to the discharging point. The current passing from the discharge point was measured by placing a plate of tinfoil of definite area beneath the discharge point, and connecting this by a carefully insulated wire, shielded by an outer metal tube connected to earth, to a Paul micro-ammeter, which was carefully screened by an earthed metal cover.

In this way it was ascertained that the current density of the discharge to which the bacteria were subjected was of the order of from 10^{-6} to 10^{-5} ampères per square centimetre.

The method of treatment of the bacteria was almost identical with that employed by Thornton, viz., a Petri dish containing a sterilised agar medium infected with the bacillus was supported on a small metal tripod, which itself stood on an earth-connected metal plate. Dish and tripod were then covered with a small bell-jar fitted with a rubber stopper, through which passed a glass tube, open at the upper end, and with a platinum wire sealed into the lower. By means of mercury, connection was made between this wire and the cable from the discharge set, and so the platinum point discharged downwards towards the Petri dish and metal plate.

1. *Discharge in Air.*—A repetition of the original experiments seemed first desirable, and accordingly cultures were exposed as described above. In 30 minutes the plates were almost cleared, subsequent incubation producing only a few colonies around the edge. In one case one side of the bell-jar was inadvertently wet, and instead of a continuous discharge, intermittent sparking down that side to the metal plate took place. The Petri dish afterwards showed sterilisation over about one-third of its area, and that on one side only. Exposure of 40 minutes or more always resulted in complete sterilisation of heavily infected dishes.

Since, then, the discharge in air was definitely deleterious to the organisms, and as it did not seem likely that ionisation effects could be the cause, the *rôle* played by the products of discharge needed investigation. These would be chiefly ozone and nitrous and nitric acids, which would be carried well on to the infected surface by the electric wind, thus providing every facility for their absorption. To test the action of these products, apart from the direct action of the discharge, a Petri dish of distilled water was exposed to the discharge under conditions identical to those obtaining in the original experiments with infected agar. The liquid was then removed and heavily infected, plate subcultures being made from it after an hour. In no case was there any development in these subcultures upon incubation, even if the water, previous to infection, were exposed to the discharge for only 20 minutes, thus confirming the idea that the products of discharge alone proved fatal.

Particular investigation of these products was now carried out by means of qualitative and quantitative tests of the distilled water after exposure. Abundance of NO_3 radical was present, as shown by the diphenylamine test, whilst addition of starch and potassium iodide solutions produced a deep blue coloration, due to nitrite or ozone, or both, also the liquid gave a distinctly acid reaction, the acidity being measured in a few cases by titration:—

No. of experiment.	Acidity as grammes of nitric acid per c.c. per hour.
1	0·00034
2	0·00072
3	0·00070
4	0·00053
5	0·00067
6	0·00060
7	0·00054

The following experiments show that solutions of about this strength of acidity, and containing nothing but nitric and nitrous acids, are capable of destroying the bacteria. A solution containing approximately 0·002 grm. of nitric acid and 0·003 grm. potassium nitrate per cubic centimetre was made up and its acidity determined. From it were made solutions corresponding respectively to

No.	Grammes of nitric acid per c.c.
1	0·0007
2	0·0003
3	0·0005
4	0·0003

Each was then infected with the bacillus, well shaken, and after an hour subcultures were made. All the plates proved sterile upon incubation, indicating the failure of the organisms to exist in such solutions. Controls with untreated distilled water were carried out simultaneously with the above experiments, and plates infected from these showed luxurious growth.

Thornton, in his paper, intimates that the criticism had been made that hydrogen peroxide might be responsible for the sterilising action of the discharge, but on discharging on to a test solution of potassium titanium sulphate, which detects minute quantities of hydrogen peroxide by the formation of yellow titanium peroxide, we found that no measurable amount of that compound was formed. Indeed, it seems unlikely that hydrogen peroxide would exist in the presence of excess of ozone, the two tending to interact with mutual reduction:—

$$H_2O_2 + O_3 \rightarrow H_2O + 2O_2.$$

2. *Discharge in Hydrogen.*—It is chiefly upon his experiments in hydrogen that Thornton bases his conclusions as to the direct instrumentality of the current in the bactericidal action. These experiments have therefore been carefully repeated, using the same form of electrode by means of which a continuous stream of hydrogen was caused to enter the discharge vessel by sweeping past the discharging point. Pains were taken to obtain the hydrogen in a comparatively pure state, since discharge in the unpurified gas resulted in the formation of a film of metallic appearance, possibly arsenic, on the object discharged upon. Therefore all hydrogen, after leaving the cylinder containing it under pressure and before being used, was passed slowly through three U-tubes containing respectively soda-lime, silver nitrate crystals and lumps of a mixture of lime and mercuric chloride, and, finally, through wash-bottles of strong sulphuric acid and potassium pyrogallate solution. In the latter, solutions of caustic potash and pyrogallic

acid were mixed, after all air had been replaced by hydrogen, by means of repeated exhaustings with a Geryk pump and refilling. When the potash and pyrogallol were allowed to mix they constituted both a test and an absorptive agent for oxygen, very little of which was present, judging from the very faint coloration of the solution.

After this treatment the hydrogen was led to the bell-jar, which for these trials was fitted with an exit delivery tube. This exit tube was connected through two more wash-bottles, the first being another test of pyrogallate, and the second merely to prevent diffusion of air back into the first. The bell-jar was rendered air-tight by sealing it to the metal plate with a stiff wax. The apparatus was then exhausted by means of a Geryk pump and slowly re-filled with purified hydrogen, this being done three times, after which the bell-jar was found to be free from oxygen. The discharge was now switched on, a stream of hydrogen being kept continually passing through the apparatus during any exposure.

Continuous discharge upon infected agar, for periods varying from 30 minutes to 2 hours, failed to produce any toxic effect, the colonies developing as quickly and in as great a number after exposure as normally. This result is the reverse of that obtained by previous investigators, Thornton, and also Foulerton and Kellas, having stated that the discharge proved fatal in hydrogen as well as in air, though the latter give no indication that any attempt was made to exclude oxygen completely. They attribute their result to the formation of hydrogen peroxide, which, by quantitative tests and subsequent trials with definite concentrations, they show to be produced in quantity sufficient to destroy the bacteria.

Thornton, on the other hand, assumes at the outset that no hydrogen peroxide was formed in his experiments, but makes no statement as to any test employed to detect it. It may have been that the compound was indeed formed, and that it was responsible for the sterilisation. Such a state of affairs is probable if the hydrogen atmosphere contain small quantities of oxygen, as was shown by some experiments of ours with such mixed atmospheres. Infected plates exposed to the discharge for 40 minutes, under such conditions, show after incubation a small clear space immediately beneath the discharging point, but the effect never approaches that obtained in air. Quantitative determinations, made by a series of comparative colour tests with the above-mentioned titanium solution, disclosed the fact that the presence of oxygen induced the formation of hydrogen peroxide in varying quantities.

No.	Total amount of H_2O_2 per hour.	No.	Total amount of H_2O_2 per hour.
1	0·000197 grm.	8	0·000099 grm.
2	0·000085 „	9	0·000114 „
3	0·000051 „	10	0·000029 „
4	0·000019 „	11	0·000174 „
5	0·000046 „	12	0·000677 „
6	0·000112 „	13	0·001459 „
7	0·000195 „		

The variation is probably due to the different amounts of oxygen present, and although quantitative determinations of the relative proportions were not made, the amounts of oxygen present in Nos. 12 and 13 were certainly larger than in the other cases.

Herein, then, may lie the explanation of the discrepancy alluded to above, the unsuspected presence of small quantities of oxygen serving to produce hydrogen peroxide in amount sufficient to cause the death of the bacteria. From the foregoing results we are led to conclude that in the destructive action of the discharge upon bacteria, the current itself plays no part, but that the gaseous products of such a discharge in air are the actively toxic agents, causing the death of the organisms, independently of the current.

Summary.

1. Electric discharge in air is fatal to bacteria exposed to its action.

2. The effect is due to the products of the interaction of the constituents of the air, namely nitric and nitrous acid and ozone.

3. Discharge in air-free hydrogen has no deleterious effect on the organisms, but the presence of small quantities of air allows the formation of a toxic substance, probably hydrogen peroxide, which again exerts a bactericidal action.

4. It, therefore, follows that electric discharges in which the current density does not exceed 10^{-5} ampère per square centimetre do not exert any directly toxic action upon micro-organisms, a result which is contrary to the statements made by some previous investigators.

Experiments on the Kidneys of the Frog. (Preliminary Communication.)

By F. A. Bainbridge, S. H. Collins, and J. A. Menzies.

(Communicated by Prof. C. J. Martin, F.R.S. Received March 27,—Read April 24, 1913.)

Introduction.

As is well known, the glomeruli of the frog's kidney are supplied with blood only by the renal arteries, whereas the renal tubules have a double supply. On the one hand, they receive blood by way of the renal portal veins; on the other hand, the efferent vessels from the glomeruli open into the capillary network round the tubules. The whole of the tubule receives blood from each of these two sources, so that the capillary network around the tubules can be fully injected either from the renal arteries or from the renal portal veins. Taking advantage of this fact it has been shown by Beddard and one of us (F. A. B.) that after complete occlusion of the glomeruli the tubules, when adequately supplied with oxygen, maintain their normal histological appearance, and may secrete urine. In the present experiments an attempt has been made to determine the function of the glomerulus and to ascertain whether the tubules possess the capacity to absorb water and solids.

Methods.

1. *Experimental.*—All the experiments were carried out on fully pithed frogs. In the earlier experiments the following method was adopted:—Ligatures were tied round the fore limbs, the heart was exposed, and the right aortic arch tied off. The ventricle and auricles were freely opened, and a cannula connected with a perfusion bottle was tied into the left aortic arch. The arterial perfusion was started at once, and the ventricle and auricles were then excised. This procedure was carried out as quickly as possible after the frog was pithed, and usually took five or six minutes. It is of importance to commence the arterial perfusion at the earliest possible moment. When most of the blood was washed out of the circulation the anterior abdominal vein was tied in two places and divided, the hind legs were tied off, and a cannula was placed in the inferior end of the anterior abdominal vein and connected with a perfusion bottle. The fluid leaving the kidneys was collected by means of a cannula placed in the beginning of the vena cava just beyond its origin from the renal veins. Finally, cannulæ were placed in the ureters. This latter operation was

much more easily carried out in the male than in the female frog, and the former were almost invariably used. Frequently the mesenteric artery was also ligatured in order to limit the extent of the perfusion.

In the later experiments the aortæ were exposed immediately above the kidneys, the right was tied off, and a cannula was placed in the left; the mesenteric artery was ligatured. Cannulæ were then placed in the vena cava just above the renal veins, the inferior end of the anterior abdominal vein (after tying off the legs) and the ureters. The testes were removed by the cautery. The advantage of this method is that the perfusion is practically confined to the kidneys, and the arterial pressure can be more readily gauged and adjusted than in the earlier experiments. The arterial perfusion through the aorta was made at a pressure of 20–24 cm. of water; the venous perfusion pressure varied from 10 to 14 cm. of water.

Solutions Used.—The following perfusing fluids were used: (1) Normal Ringer's solution (NaCl 0·65 per cent., KCl 0·02 per cent., $CaCl_2$ 0·03 per cent.); (2) Hypotonic or hypertonic solutions of sodium chloride containing also potassium chloride 0·02 per cent. and calcium chloride 0·03 per cent. —these are subsequently termed hypotonic or hypertonic Ringer's solution; (3) Hypotonic or hypertonic Ringer's solution with the addition of 0·1 or 0·2 per cent. sodium sulphate (anhydrous). The solutions were fully oxygenated and were filtered before being put into the perfusion bottle. Frequently oxygen was also bubbled through the perfusing fluid in the bottle. The perfusion bottles were provided with a Mariotte tube.

2. *Physical.*—The greater part of the work which required analytical determinations of the materials used was carried out by means of the refractometer. The instrument used was of the Pulfrick type without water cooler. To enable the instrument to work with less than one drop of liquid, a small flat bottom tube was placed in the refractometer cup. Between the top of the prism and the bottom of the flat bottom tube there was a thin film of the liquid tested; in the tube was a little water with a thermometer. As neither the Pulfrick angles nor the corresponding indices convey much meaning in the present communication, all the results are returned as having a refractive index equal to a solution of sodium chloride of some special strength. As has been shown before,* the refractive index of solutions is proportional to their concentration. The refractive index of water on the particular instrument used is at $20° C. \pm 1° = 67° 12{\cdot}7' \pm 0{\cdot}8'$ with a probable error of one determination of $\pm 0{\cdot}65'$. The value of NaCl is $1' = 0{\cdot}0443$ per cent. NaCl, and the determinations of strength of sodium

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* B. Walter, 'Ann. Phys. Chem.,' vol. 38, p. 107; 'Journ. Chem. Soc.,' 1890, A, p. 202.

chloride between ½ and 2 per cent. of pure NaCl showed a probable error of ± 0·02 per cent. NaCl in such solutions. The actual figures given in the communication must be considered as having that degree of error. Although other salts, as KCl, $CaCl_2$, were used, the amounts taken were too small to introduce any appreciable error on this account. Since the solutions used were very nearly solutions of one single chemical substance, the refractometer readings, like specific gravity, give the molecular concentration of the solution.

3. *Histological.*—Immediately after each experiment the kidneys were removed and placed in a fixing solution. This was generally alcohol, but in some cases formalin (10 per cent.) and in others Flemming's fluid was used. After being hardened the kidneys were embedded in paraffin and a series of sections was taken from the middle of each kidney. When the blood-vessels had been perfused with a mercuric salt and ammonium sulphide the sections were mounted unstained. In other cases stains were used, generally hæmatoxylin and eosin. In one case complete serial sections were made of the pair of kidneys.

Injections of the blood-vessels were also made as described below and serial sections prepared.

Results.

Histological.—The validity and significance of most of the experiments to be described rests upon the proof that the whole of the capillary network round the tubules normally receives blood both from the efferent vessels of the glomeruli (that is, by way of the arterial system) and from the renal portal vein. In order to demonstrate this we have injected a number of kidneys on the one hand from the aorta, after ligature of the renal portal veins, and on the other hand from the renal portal veins after occlusion of the arterial circulation. The injections were made from a perfusion bottle under a pressure approximately equivalent to the normal blood-pressure in the frog's kidney, namely 20–24 cm. of water for the arterial perfusion and 10–12 cm. of water for the venous perfusion. In all the experiments the venous outflow was unobstructed. The fluids used consisted of (*a*) Berlin blue and (*b*) carminate of ammonia in Ringer's solution. A few double injections were made, carmine by the arteries and Berlin blue by the renal portal vein. One gelatin double injection was also made, but in this case the pressure used was obtained by means of an injection syringe and pressure bottle and was higher than usual. It was found that whether the single injection was made by the artery or by the renal portal vein the whole of the intertubular capillary network appeared to be injected. In the case of the double injections the glomeruli and efferent vessels were filled with the arterial injection fluid, whereas the intertubular capillaries showed, some the arterial fluid, some the venous, and some a mixture of both. We confirmed Beddard's observation that the perfusion of Berlin blue solutions at a low pressure through the renal portal vein leaves the glomeruli completely uninjected. We found, however, that if the venous perfusion were made under an abnormally high pressure (*e.g.* 35 cm. of water) the coloured solution eventually made its way into some, at least, of the glomeruli. It was further shown by arterial perfusion with Berlin blue that the glomeruli will withstand a perfusion pressure of at least 40 cm. of water.

Other evidence that the renal portal blood supplies the whole of the tubules was obtained by perfusing 1/10,000 mercuric chloride at 10 cm. pressure for three to five minutes through the renal portal vein, and then perfusing through the vein under the same pressure first saline solution for a few minutes and then a very weak solution of ammonium sulphide in saline solution. The whole of the tubules showed a deposit of mercuric sulphide while the glomeruli remained free from it in the vast majority of cases. It seems clear, therefore, that a poison reaching the kidney by way of the renal portal vein will come in contact with the whole of the tubules and yet leave the glomeruli practically or quite intact. The only risk is that a diffusible poison, if not quickly rendered inert or washed out of the kidney, may gradually reach the glomeruli by direct diffusion. This risk is minimised by having a simultaneous arterial perfusion and by using the poison in a concentration which is just adequate to kill the tubules when brought into immediate contact with them. Whether the arterial blood supply alone provides the tubules with a sufficiency of oxygen has yet to be determined by experiments on the living animal. It is known, however, that the venous supply alone will maintain their nutrition in the living frog provided that the frog is kept in an atmosphere of oxygen. There can be little doubt that in these experiments, in which the perfusing fluid was fully oxygenated, both the supply of oxygen to the tubules in a venous perfusion and that to the glomeruli in an arterial perfusion were amply sufficient to maintain their vitality so long as the rate of perfusion remained normal.

Experimental.—In most of the experiments to be described the perfusion was made with normal or hypotonic Ringer's solution, and the experiments made with hypertonic Ringer's solution will only be referred to incidentally.

(1) *The Normal Kidney.*—The rate of arterial perfusion varies considerably in different experiments, doubtless as a result of the varying tone of the glomerular vessels, and more particularly the efferent vessels; it is apt also to decrease in the course of a single experiment. Since the oxygen supply

to the kidneys varies directly with the perfusion rate, a slow perfusion leads to an inadequate oxygen supply to the glomeruli or tubules.

The amount of fluid escaping from the renal veins on an arterial perfusion alone varied from 20 to 60 c.c. per hour in different experiments; an average rate was about 30 c.c. per hour. On a venous perfusion alone the rate of perfusion was more constant and averaged 60–70 c.c. per hour.

Flow of Urine.—The amount of urine obtained from the normal kidneys on an arterial perfusion alone varies directly with the rate of perfusion, and under favourable circumstances as much as 1·5 c.c. may be obtained in less than an hour. The concentration of the urine is almost always notably less than that of the perfusing fluid when the latter is hypotonic Ringer's solution; if the kidneys are perfused with normal Ringer's solution the urine may be isotonic with, but is usually hypotonic to, the perfusing fluid.

The urine obtained on a simultaneous arterial and venous perfusion does not, so far as we could determine, differ in amount from that obtained on an arterial perfusion alone; a simultaneous arterial and venous perfusion, however, seems to be more conducive to the formation of a very dilute urine than is arterial perfusion alone.

Table I.—Urine from Living and Dead Kidneys.

Experiment.	Concentration.				
	Perfusing fluid.	Urine from normal kidneys.		Urine from dead kidneys.	
		per cent.	per cent.	per cent.	per cent.
1	0·59 per cent. NaCl	(*a*) 0·42	(*b*) 0·46		
2	0·57 „ „	(*a*) 0·40	(*b*) 0·38		
3	0·55 „ „	(*a*) 0·33	(*b*) 0·40		
4	0·53 „ „	0·25			
5	0·42 „ „	0·30		0·40	
6	0·72 „ „	(*a*) 0·55	(*b*) 0·49		
7	0·83 „ „	(*a*) 0·73	(*b*) 0·68	(*a*) 0·83	(*b*) 0·83

The letters (*a*) and (*b*) refer to successive samples of urine.

In Experiment 7 the perfusing fluid contained 0·1 per cent. Na_2SO_4; in the others the perfusing fluid was simply normal or hypotonic Ringer's solution.

On a venous perfusion alone no urine was secreted with any of the perfusing fluids used. In some of these experiments the arterial circulation was excluded by tying the aortic bulb at the outset and allowing the glomeruli to become infarcted; in others, the glomeruli had previously been perfused with Ringer's solution and the arterial perfusion shut off.

(2) *The Dead Kidney.*—The vitality of the kidney was destroyed by perfusing it through the aorta either with weak (1/10,000) corrosive sublimate or with boiled Ringer's solution. After the former procedure the arterial perfusion with Ringer's solution was resumed under normal pressure. The rate of perfusion and the amount of urine obtained were always much less than in the normal kidney, and sometimes, with a very slow perfusion rate, the flow of urine entirely ceased. The urine was usually isotonic with, but occasionally hypertonic to, the perfusing fluid, the latter only in experiments in which the formation of urine was extremely slow and scanty.

(3) Since the urine obtained in all these experiments comes solely from the glomeruli (the tubules secrete no urine), it is natural to suppose that the difference in the character of the urine formed by the intact and dead kidneys respectively depends upon one of two causes. On the one hand, the glomeruli may normally form by filtration a urine which is isotonic with the perfusing fluid, and the absorption of salt may be effected by the tubules as the glomerular filtrate passes along them. On the other hand, the tubules may possess no absorptive power for sodium chloride or other salts, and the glomeruli may possess the capacity to secrete a hypotonic urine. In attempting to decide between these two possibilities, two methods have been used.

(*a*) The tubules were poisoned by perfusing 1/10,000 mercuric chloride through the renal portal vein for three to five minutes, and then Ringer's solution was perfused for a few minutes through the renal portal veins, to wash away the mercury in the blood-vessels. The arterial perfusion of oxygenated Ringer was maintained throughout the experiment. The urine obtained both before and after the poisoning of the tubules was examined, and at the end of the experiment the mercury was fixed in the tubule cells by perfusing dilute ammonium sulphide through the renal portal veins, and the kidneys were examined histologically. It was found in most of the experiments that the glomeruli remained free from mercury, and that mercuric sulphide was present in the whole of the tubules. This was also the case in control experiments in which the mercury was fixed by ammonium sulphide immediately after it had been perfused through the renal portal vessels. Experiments in which mercury was present in the glomeruli were rejected. The following protocol illustrates the character of these experiments:—

Protocol I.—Pithed Male Frog. Cannulæ in left aorta, origin of vena cava, anterior abdominal vein and ureters. Hind legs tied off. Mesenteric artery and right aorta ligatured and testes removed.

	Time.	Fluid escaping by v. cava.	Urine.	Concentration of urine.
		c.c.		
Simultaneous arterial and venous perfusion oxyg. Ringer's solution.	3.15–3.30	22	3.10–3.30 R. K. 0·1 c.c.	0·33 per cent. NaCl.
	3.30–3.45	27	3.30–3.40 R. K. 0·2 ,, L. K. 0·2 ,,	0·32 ,, ,, 0·29 ,, ,,
	3.45–4.0	21	3.40–3.57 R. K. 0·2 ,, L. K. 0·2 ,,	0·41 ,, ,, 0·40 ,, ,,
3.58–4.4. Perfused 1/10,000 $HgCl_2$ through renal portal veins. Arterial perfusion maintained. Then perfused Ringer's solution for 5 minutes through renal portal veins, to wash the mercuric chloride out of the vessels.				
Simultaneous perfusion, art. oxyg., venous non-oxyg., but containing 0·1 p. c. caffein. A. press. 24 cm. V. 12 cm. Ringer = 0·53 per cent. NaCl	4.0–4.30	43	4.0–4.30 R. K. 0·1 c.c. L. K. 0·1 ,,	0·53 per cent. NaCl 0·52 ,, ,,
	4.30–5.0	48		
Finally perfused weak ammonium sulphide through renal portal vein. Kidneys cut in serial section; no mercury observed histologically in the glomeruli.				

The venous perfusion of corrosive sublimate causes considerable vaso-constriction, and the efferent vessel from the glomerulus which ends in the tubular capillary network appears to be particularly affected. This, at least, is our interpretation of the extremely slow perfusion through the glomeruli which is met with under these circumstances and which is associated with a lessened flow of urine. We have attempted to overcome this vaso-constriction by perfusing through the renal portal veins Ringer's solution containing a trace of acetic acid or 0·1 per cent. caffein sodium benzoate, but without complete success. We have obtained, however, a rate of perfusion through the glomeruli which was adequate to maintain their vitality, and in some experiments was for a time equivalent to that of the normal kidney, although it eventually became very slow. The employment of 1/10,000 mercuric cyanide caused less vaso-constriction, but also less definite histological evidence of the complete poisoning of the tubules. The results of a number of these experiments are shown in Table II.

Table II.—Effect of Poisoning the Tubules with Corrosive Sublimate. The perfusing fluid was in all cases hypotonic Ringer's solution.

Exp.	Concentration of urine.		Concentration of perfusing fluid.	Histological result.
	Normal kidneys.	Poisoned kidneys.		
	per cent.	per cent.		
1	0·33, 0·29, 0·40	0·53, 0·52	0·53 per cent. NaCl	Glomeruli intact, tubules all show Hg.
2	0·48, 0·43	0·60	0·58 " "	
3	0·30	0·40	0·42 " "	
4	0·47, 0·40	0·51	0·57 " "	

Different figures represent separate samples of urine.

(*b*) The kidneys were first perfused with oxygenated Ringer's solution simultaneously by the aorta and renal portal veins. Then the glomeruli were perfused with boiled Ringer's solution in order to kill them, while the tubules were still receiving by the veins an adequate supply of oxygen. A typical experiment is shown in the following protocol:—

Protocol II.—Pithed Male Frog. Cannulæ in left aorta, anterior abdominal vein, inferior vena cava and ureters. Mesenteric artery and right aorta ligatured. Hind legs tied off. Simultaneous arterial and venous perfusion. Arterial pressure 24 cm., venous 12 cm. Molecular concentration of Ringer's solution (refractometer) = 0·55 per cent. NaCl. Ringer's solution contained 0·5 per cent. NaCl, 0·02 per cent. KCl, 0·03 per cent $CaCl_2$ in distilled water.

Time.	Escape from vena cava.	Urine.	Concentration of urine.
	c.c.		
11.47–12.4	31	11.40–12.10 R. K. 0·1 c.c. L. K. 0·15 "	0·40 per cent. NaCl 0·46 " "
12.4–12.28	51		
12.28–12.47	37	12.10–12.48 R. K. 0·15 " L. K. 0·15 "	0·33 " " 0·34 " "
12.47–1.17	33	12.48–2.15, both kidneys 0·1 c.c.	0·36 " "
1.17–1.47	27		
1.47–2.17	23		
2.17–2.47	25		
12.50 onwards.	Glomeruli perfused with boiled Ringer's solution.		

It will be noticed that the cutting off of the oxygen supply to the glomeruli lessened the rate of arterial perfusion and also the amount of urine formed. The general result of a number of such experiments is shown in the following table:—

Table III.

Expt.	Concentration of urine.		Concentration perfusing fluid.
	Normal kidney.	Glomeruli killed.	
1	(*a*) 0·48 per cent. NaCl (*b*) 0·45 „ „	(*a*) 0·45 per cent. NaCl (*b*) 0·45 „ „	0·56 per cent. NaCl
2	(*a*) 0·43 „ „ (*b*) 0·34 „ „ (*c*) 0·33 „ „	0·36 „ „	0·55 „ „
3	(*a*) 0·48 „ „ (*b*) 0·38 „ „	(*a*) 0·42 „ „ (*b*) 0·43 „ „	0·50 „ „
4	(*a*) 0·46 „ „ (*b*) 0·36 „ „	(*a*) 0·48 „ „ (*b*) 0·48 „ „	0·53 „ „

In all the experiments the perfusing fluid contained sodium chloride plus 0·02 per cent. KCl and 0·03 per cent. $CaCl_2$. The letters (*a*) and (*b*) refer to separate samples of urine.

(*c*) *Caffein.*—Barcroft and Straub have shown that caffein sodium benzoate greatly diminishes the consumption of oxygen by the mammalian kidney, and they used it for the purpose of poisoning the renal tubules. We have carried out a number of experiments to determine the action of caffein on the frog's kidney. In our early experiments 1 per cent. caffein sodium benzoate was perfused through the renal portal veins for five minutes, the arterial perfusion of oxygenated Ringer's solution being simultaneously maintained; the collection and examination of the urine was not begun until from one to two hours after the perfusion of the caffein, and it was found to be hypotonic to the perfusing fluid. At that time we believed that caffein (in the dose given) permanently poisoned the tubules, and we therefore regarded the glomeruli as capable of secreting a hypotonic urine. Further experiment seems to show, however, that the poisoning effect of caffein is merely temporary. The immediate effect is to render the urine isotonic with the

Table IV.—Action of Caffein Sodium Benzoate.

Expt.	Concentration of fluid in terms of NaCl.			
	Perfusing fluid.	Normal urine.	Urine after poisoning tubules with caffein	
			Immediately after.	1–2 hours after.
	per cent.	per cent.	per cent.	per cent.
1	0·53	0·25	0·54	—
2	0·55	0·40	0·55	—
3	0·51	—	—	0·22, 0·30

perfusing fluid, but this effect gradually passes off and, unless more caffein is injected, the urine once more becomes hypotonic to the perfusing fluid. We found that if 0·1 per cent. caffein were continuously perfused through the renal portal veins the urine remained isotonic with the arterial perfusing fluid throughout the experiment. Unfortunately it is impossible to trace the caffein histologically into the tubule cells, and we do not know whether it attacks the whole length of the tubules or not. We regard these results, therefore, as merely subsidiary to and confirmatory of those in which the tubules were poisoned with mercury.

The formation of a urine which is hypertonic to the perfusing fluid has occasionally been observed after poisoning the tubules with corrosive sublimate and even in the dead kidney. It occurs only when the formation of urine is extremely slow ; we are not yet satisfied as to its significance.

Summary and Conclusions.

When the frog's kidneys are perfused through the aorta and the renal portal veins with oxygenated normal or hypotonic Ringer's solution, the urine formed is hypotonic to the perfusing fluid and is derived entirely from the glomeruli, since the tubules secrete no urine under these circumstances. When the tubules are poisoned with corrosive sublimate or (temporarily) with caffein, the urine becomes isotonic with the perfusing fluid. On the contrary, if the glomeruli are killed by the arterial perfusion of boiled Ringer's solution, while the tubules still receive an adequate supply of oxygen through the renal portal veins, the urine formed continues to be more dilute than the perfusing fluid. These results suggest, first, that the glomeruli form by filtration a urine isotonic with the perfusing fluid, and, secondly, that during the passage of the glomerular filtrate down the tubules sodium chloride is absorbed by them. Whether any water is also absorbed we do not know.

The expenses of this research have been defrayed by a grant from the Government Grant Committee of the Royal Society.

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The Effect of the Lability (Resilience) of the Arterial Wall on the Blood Pressure and Pulse Curve.—II.

By Leonard Hill, F.R.S., and Martin Flack.*

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(From the Physiological Laboratory, London Hospital Medical College, London Hospital Research Fund.)

In a paper published in the 'Proceedings of the Royal Society,' 1913, B, vol. 86, p. 180, Russell Wells and Leonard Hill brought forward evidence to show that the nature of the arterial wall has an important effect in modifying the conduction of the pressure waves from the heart to those arteries where the pulse is usually explored, such, for example, as the radial, where sphygmograms are recorded and readings of arterial pressure taken with the sphygmomanometer. They concluded that the conduction depends on the greater or less "resilience" of the arterial wall, using the term "resilience" to express "the ease with which an elastic tube distends with a rise and recoils with a fall of pressure of the contained fluid"; thus a rubber tube with a wall of 0·2 mm. thick is more "resilient" than one with a wall 0·4 mm. thick, the thinner, more "resilient" tube yields with the rise and recoils with the fall of pressure more than the "harder," thicker-walled tube. A glass tube, in this sense, has no resilience, and the same may be said of rubber pressure tubing. As the arterial wall contains muscle, its "resilience" will be altered by a more or less contracted state, also since the degree of contraction and "resilience" may vary locally it is to be expected that the curve of blood-pressure may likewise vary, *e.g.* in the brachial and in the femoral arteries. We have found this to be the case under certain conditions, namely, in cases of aortic regurgitation.† In such cases the systolic pressure reading for the leg is much higher, 100 mm. or more, than in the arm arteries. Also in normal men a difference in the systolic pressure in the two radial arteries may be observed when the heart is made to beat forcibly by a short period of hard exercise and after one elbow has been placed in hot and the other in cold water. The artery relaxed by heat gives the lower systolic pressure.

Russell Wells constructed a schema by means of which a known rhythmically changing pressure could be passed (1) through rubber tubes of the same calibre, but varying thickness, *e.g.* 0·8, 0·4, 0·2 mm., (2) through various lengths of the same tube, (3) through the same tube and same length of tube, but with increasing amplitude. With an entering pressure of 160 mm. systolic and of 40 mm. diastolic, a curve taken with the Hürthle manometer from the 0·8 mm. tube had all the characters of a " low " pressure sphygmogram—great amplitude, sharp rise and fall and very well-marked dicrotic wave; while with exactly the same entering pressure the curve taken from the 0·2 mm. tube took on all the characters of a " high " pressure sphygmogram—slow rise, flat top, slow fall and slightly-marked dicrotism. Using the same tube, it was found that the higher the pressure and the more the " resilience " of the tube was brought into action, the nearer together were the diastolic and systolic pressures at the end of the tube. A length of 30 cm. of 0·2 mm.-thick tube with an entering pressure of 78 mm. Hg diastolic and of 148 mm. Hg systolic gave an almost continuous pressure of 104 mm. diastolic and 107 mm. systolic at its farther end. The same length of 0·8 mm.-thick tube gave a much more discontinuous pressure at its farther end. Lengthening a given tube had a like effect, approximating the diastolic and systolic pressures at its farther end, *e.g.* by increasing the length of a 0·8 mm. tube from 15 to 30 cm. the difference between systolic and diastolic pressures was diminished from 66 to 44 mm.

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* During tenure of Eliza Ann Alston Research Scholarship.

† L. Hill, with Martin Flack and W. Holtzmann, 'Heart,' 1909, vol. 1, p. 73; L. Hill and R. A. Rowland, 'Heart,' 1912, vol. 3, p. 222.

The use of the word " resilience " in the sense given above is not in accordance with the meaning given to this word by the physicist, and in the discussion which followed the reading of the above paper it was suggested by the President of the Royal Society that the word " lability " might be suitable. We propose to adopt this word and thus free ourselves from the charge of ascribing to " resilience " an equivocal significance. By the " lability " of an artery, then, we mean the ease with which it distends with a rise and recoils with a fall of pressure.

We have investigated the effect of the " lability " of arteries on the pressure curve, (1) by means of a simple schema, (2) by interpolating a length of artery between a Hürthle manometer and the carotid artery when recording the blood-pressure curve in the living animal. The schema consists of a piece of rubber pressure tubing, connected at one end to a Hürthle manometer and branching into two channels at the other end by means of a T-piece. A short length of rubber pressure tubing forms the channel on one side, and an equal length of artery that on the other side. The two channels are connected by another T-piece to a short length of rubber pressure tubing, which is closed at its farther end. This tube is rhythmically pulsed between the thumb and finger, the whole schema being filled with water to a pressure equal to that of the normal arterial pressure. Clamps are arranged so that, in turn, either the rubber pressure tubing or the artery is made to conduct the pulse to the manometer. We rhythmically pulsed the tube as hard as possible between thumb and finger, so that the pressure curves produced were made of approximately equal amplitude.

Fig. 1 shows the curve obtained—A, when 5 inches of rubber pressure tube conducts the pressure-wave to the manometer; B, when the conduction is by 5 inches of femoral artery (human), all the branches of which have been tied so that there is no leak. The "lability" of this length of artery has a very great effect, as is seen by the diminution of the systolic wave and the absence of "overswing" secondary waves. Fig. 2 shows—A, the curve obtained from the carotid artery of a cat, conducted to the manometer by a 23-cm. length of pressure tubing; B, when the manometer is connected by a 23-cm. length of cat's artery, made up of the aorta together with the part of one carotid and one femoral artery. The arrangement was such that the pulse curve could be transmitted alternately by the rubber pressure tube or by the artery. Fig. 3 shows the effect of transmitting the pressure curve from the carotid of a cat through—A, 8 cm. of rubber pressure tubing; B, through 8 cm. of excised cat's carotid. Fig. 4 shows the effect of transmitting the curves—A, through 6 cm. of rubber pressure tubing; B, through 6 cm. of cat's carotid. Fig. 5 shows that, after hardening the 6-cm. length of carotid artery in alcohol, the same result is obtained as with rubber pressure tubing. Fig. 6 demonstrates the effect of 4·5 cm. of cat's carotid (B) on the conduction of the pressure curve. Even this short length notably alters the curve, particularly in diminishing the dicrotic wave.

Fig. 7 shows the effect of connecting one end of an 8-cm. length of carotid artery, closed at the other end, to the rubber pressure tube through which the pressure curve was being conducted. This brings down the amplitude and approximates the systolic and diastolic pressures just as effectually as if the conduction were wholly through the same length of carotid. Fig. 8—A shows the curve taken from the right and left carotid of a cat. On the right side the cannula was inserted as near the aorta as possible, while on the left side the cannula was inserted as far from the aorta as possible. The dicrotic wave is much less marked in the tracing taken from the longer length of carotid. B shows tracings taken from the right and left carotid, both being as short as possible. There is no noteworthy difference. We give this tracing as a control, to show that the conduction by the length of artery is the factor which makes the difference in A. Fig. 9 shows the pressure curve of the cat taken—A, from the long length of left carotid still embedded in the tissues; B, from the short length of right carotid. In A, the dicrotic wave is less evident, and the curve has a flatter top, but the difference is less than in fig. 8.

Fig. 1.

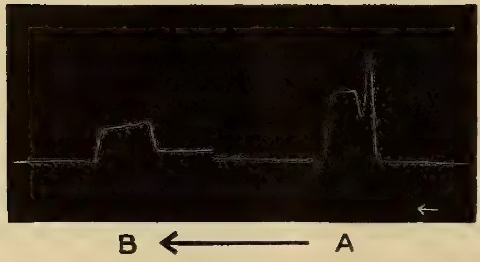

Fig. 2.

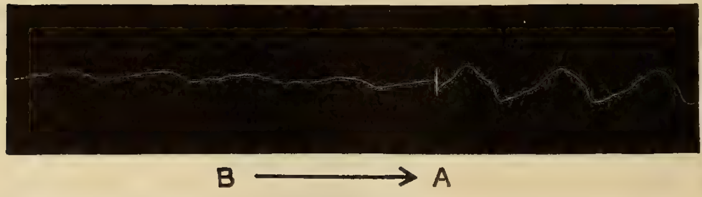

Fig. 3.

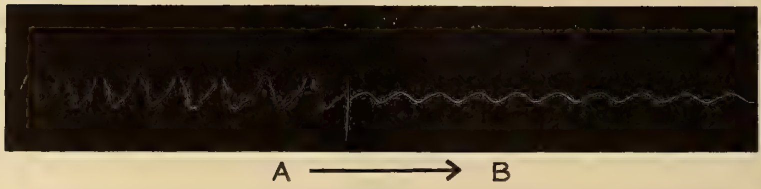

Fig. 4.

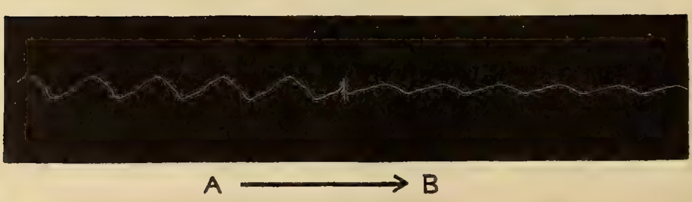

Fig. 5.

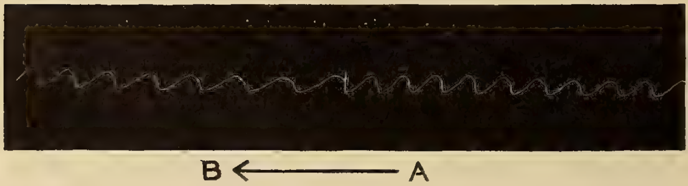

Fig. 6.

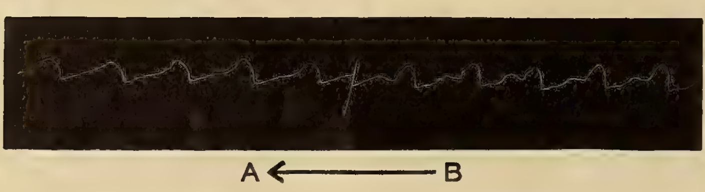

Fig. 7.

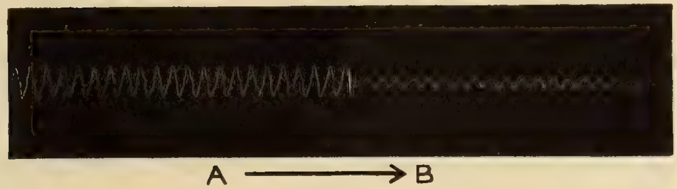

Fig. 8.

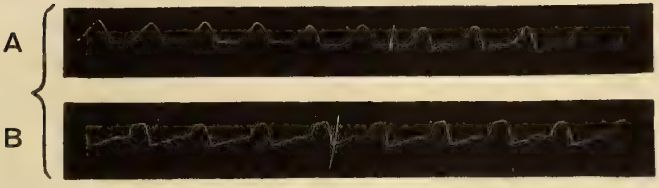

Fig. 9.

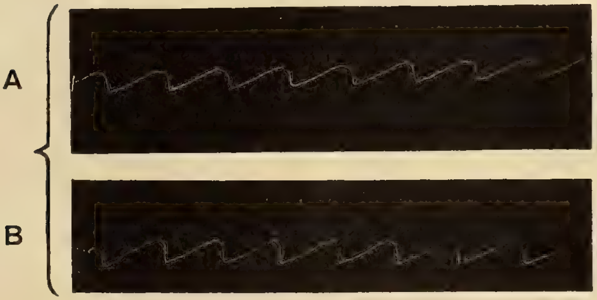

Fig. 10.

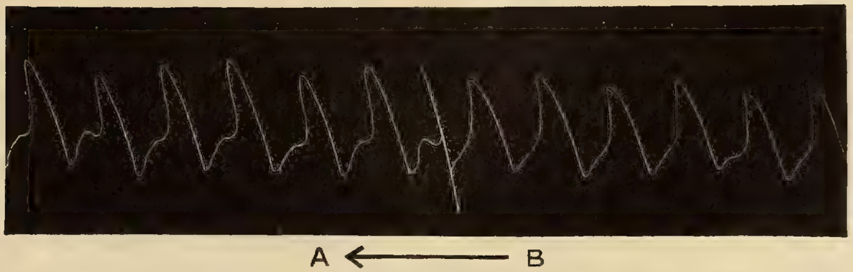

Fig. 10 shows the pressure curve taken from the carotid artery of a dog—A, through the rubber pressure tubing; B, through 5 inches of human femoral artery. The greater approximation of systolic and diastolic pressure and the diminution of the dicrotic wave is evident.

These results confirm our previous conclusion that the nature—"lability"—of the arterial wall notably affects the conduction of the pressure wave, and therefore both the form of the sphygmogram and the readings of pressure obtained with the sphygmomanometer. Much of the systolic force of the heart is stored as potential energy in the distension of labile large arteries, to be given up again on their recoil during diastole; a part is spent in overcoming their resistance to distension; the greater the lability the less will be the amplitude of the systolic wave which reaches the peripherally placed arteries; the higher also will be the diastolic wave, the closer the approximation of the diastolic and systolic pressures, the less marked the dicrotic wave.

It follows from these considerations that, supposing the force of the heart remains constant, what has been termed a "high" or a "low" pressure form of sphygmogram does not depend only on the resistance in the arterioles, but may be obtained according to the greater or less "lability" of the conducting arteries—*e.g.* aorta—subclavian—brachial—radial. So, too, will the readings of systolic and diastolic pressure vary with the "lability" of the conducting arteries. Observations on cases of aortic regurgitation have shown us that the systolic readings may be 100, even 150, mm. Hg higher in the leg than in the arm arteries. This great difference is entirely due to conduction modified by the "lability" of the arteries. The arm arteries in such cases are more "labile" than the leg arteries—the latter are contracted, "harder," more rigid, and conduct the systolic wave from the aorta with far less diminution of force. This is to ensure a circulation through the capillaries of the leg, to compensate for the great fall of diastolic pressure. By local modification of the contractile state of the arteries either the full hammer-like stroke of the heart may be delivered to the capillary vessels with a wide variation between the systolic and diastolic pressures, or a more uniform pressure be conveyed with the systolic variations of pressure approximated.

In reading the systolic or diastolic pressure by means of the sphygmomanometer we read, not the actual pressure produced by the heart, but this pressure as conducted by the arteries to that artery selected for observation. In the normal young man, placed in the recumbent posture, the arteries so conduct, that approximately the same readings are obtained in the arm and in the leg arteries. The contraction of the muscular coat of the arteries is controlled so as to effect this. In cases of aortic regurgitation the conduction is widely different. A similar difference may also pertain in conditions of functional activity, *e.g.* the leg arteries may be more contracted and give higher readings of systolic pressure after running up a flight of stairs. High readings of systolic pressure do not necessarily indicate any greater systolic force exerted by the heart. They may indicate, and probably often do indicate, less "lability"—arteries held in the contracted state so as to conduct the systolic wave with almost undiminished force. The character of pulse curves, taken either with a Hürthle or other spring manometer, placed in direct communication with an artery, or by means of the sphygmograph, depends very largely on the "lability" of the conducting arteries. It is arterial "lability," not reflection of waves, which modifies the form of the pulse curve taken in different arteries. While the pressure waves produced by the heart may remain the same, the form of the sphygmogram may be altered, and what has been termed a "high" or a "low" pressure curve may be produced by variation in the "lability" of the conducting arteries. The wall of an artery is supported by the surrounding tissues and skin, the whole being permeated with blood; it will be a matter for further consideration as to how far the lability is affected by the condition of the surrounding tissues. Comparison of figs. 8 and 9 shows how large a part the tissues normally take in supporting the arteries.

On the Probable Value to Bacillus coli *of " Slime" Formation in Soils.*

By Cecil Revis.

(Communicated by Sir J. R. Bradford, K.C.M.G., Sec. R.S. Received April 8,—Read April 24, 1913.)

During the course of an investigation into the causes of variation in the physiological activity of *Bacillus coli*, a number of experiments were started, in which soils, either virgin or mixed with cow dung or human excreta, were inoculated with cultures of *B. coli*, together with cultures of various soil organisms so different from the colon organism that they could not be mistaken for it on plating out. The requisite quantity of soil was placed in a layer about ¾-inch deep in large flat litre-bottles, and the cultures were added in the form of emulsions in physiological salt solution, made from agar slopes. Sufficient water was also added to make the soil visibly moist. The bottles were closed with cotton-wool plugs and kept at ordinary room temperature in the dark. Controls which were inoculated with all the organisms except the *B. coli* were started at the same time. The soils were examined from time to time by withdrawing about 5 grm. by means of a sterile tube, shaking this up with 50 c.c. of sterile water, spreading plates directly on to ordinary agar and incubating at 20° C.

It was found very difficult to isolate the *B. coli* in this way because of the rapid and expansive growth of the other organisms present, and because the experimental organism did not usually grow in a typical manner, but in large watery colonies, which were at first not recognised as *B. coli*, and were also soon involved with other growths on account of their spreading nature. It was therefore necessary to employ the usual method of preliminary inoculation into bile-salt glucose broth, followed by plating out into ordinary (+I), or bile-salt agar. In this way *B. coli* was always readily isolated, but the tendency to form large, moist, slimy colonies was still marked, a characteristic to which I have directed attention before.

The results were not of any great interest from the point of view of variation. From time to time, during the first 18 months of the investigation, apparently typical *coli* were isolated which refused to grow in peptone water or to attack any of the test substances. In many cases, the original culture from the plate failed to attack dulcitol and sometimes mannitol, but these failures were not of a permanent character. Towards the end of the experiment quite typical organisms only were obtained. The necessary use of bile-salt broth possibly is adverse to the separation of atypical organisms in the presence of a preponderance of typical forms. There was not apparently during the whole course of the experiment (which lasted three years) any marked diminution of the original *B. coli*, as it could be recovered in all cases from at least 0·00001 grm. of the soil.

The remarkable point of the investigation lies, however, in the fact that throughout the course of the experiment no further addition of water was made to the flasks. The control flasks, which did not contain *B. coli* (though all the other soil organisms were present), dried up within a few months of the start. In all the flasks which contained *B. coli* not only did the flasks retain their moisture for three years, but during the first 12 months of the experiment had evidently taken up large quantities of moisture from the atmosphere, and in one or two instances the soil became completely water-logged.

It seems evident that this extraordinary behaviour is connected with the *B. coli*, and in view of the fact, which I have constantly noticed, that this organism can easily produce "slime" (without the presence of sugar), and that when grown in this manner in soil it certainly does so, it seems reasonable to attribute the water-absorption of the soil to this curious property. These results possibly give at the same time some explanation of the well-known power of many organisms which occur in soil, especially the "nodule" bacteria, to form "slime."

The viability of *B. coli* for such a long period is also remarkable, but cannot, of course, be taken as true for ordinary soils, as there are bactericidal influences at work in such, which have been destroyed by the initial sterilisation necessary in these experiments.

Variation in Bacillus coli.—*The Production of Two Permanent Varieties from one Original Strain by means of "Brilliant Green."*

By Cecil Revis.

(Communicated by Sir J. R. Bradford, K.C.M.G., Sec. R.S. Received April 8,—Read April 24, 1913.)

In a former communication[*] it was noted that a profound change in physiological activity could be brought about in some strains of *Bacillus coli* by growth in the presence of Malachite Green. This change consisted in the complete loss of power to produce gas in the usual test media, the action ceasing at the acid stage.

Similar experiments have been carried out using Brilliant Green in place of Malachite Green, and many interesting results have been obtained. The following is of particular interest in that two permanent varieties arose from one original organism under precisely similar circumstances. The method employed was exactly the same as in the former experiments. The culture used was repeatedly plated until it was evident that the final culture used had arisen from one single organism. This procedure is undoubtedly better and less liable to error than some of the more complicated methods which have been proposed (*cp.* Eisenberg[†]).

The properties of the original culture were:—

Milk	Coagulated	in 48 hours.
[‡]Lactose peptone water	+ + +	" "
Sucrose "	——	
Adonitol "	——	
Dulcitol "	+ + + +	on 5th day.
Inulin "	——	
Glucose "	+ + +	in 48 hours.
Salicin "	+	on 5th day.
Mannitol "	+ + + +	in 48 hours.

This selected culture was then inoculated into nutrient broth (+I) containing 0·004 per cent. of Brilliant Green. Two different inoculations were started in order to provide a control for any changes which might occur. This procedure has, however, been found subsequently to be of little avail, as in many cases in which two inoculations from the same cultures have been so started they have not behaved in the same manner under the same circumstances. In this particular case one culture experienced much greater difficulty in growing in the presence of Brilliant Green and eventually died out. The other which survived was finally plated out, when it had been trained to grow in broth containing 0·05 per cent. of the dye, and the organism was then developing well in it. The plating out was made on ordinary nutrient (+I) agar and the plates were kept at 20° C.

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[*] Revis, 'Roy. Soc. Proc.,' 1912, B, vol. 85, p. 192.

[†] Eisenberg, 'Cent. für Bakt.,' 1912, Abt. I, vol. 63, p. 305.

[‡] The positive sign indicates the production of *acid* and *gas* in the test medium, using 5 c.c. medium and a gas tube $\frac{3}{16}$ inch diameter. The signs +, + +, + + +, and + + + + indicate $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, and $\frac{3}{4}$ inch of gas in the tube. No change, thus ——.

Two types of colony arose:—(A) very small, (B) large and arborescent. Both varieties grew rather slowly at 20° C. on the plates, as did also subcultures from them. They were tested in the usual peptone water media with the following results:—

(A)	Milk	Acid in 7 days, but no coagulation at all.
	Lactose peptone water	+ + in 48 hours.
	Sucrose „	——
	Adonitol „	——
	Dulcitol „	*A., sl. G., about the 20th day.
	Inulin „	——
	Glucose „	+ + in 48 hours.
	Salicin „	A., sl. G., on the 7th day.
	Mannitol „	+ in 48 hours.

(B) was very similar, but milk coagulated in 48 hours, dulcitol was not attacked at all, and only A., sl. G. occurred in mannitol.

It is noteworthy that the organism which showed the greater vitality (judged by growth on agar) had suffered the greater loss of fermentative power. This has been found to be the rule in other cases. Both of these organisms (A and B) were carried on in Brilliant Green broth (0·05 per cent.) but (A) soon succumbed, while (B) grew as well as before. This will now be called Culture B.

The original culture in Brilliant Green broth from which these (A and B) were obtained was kept going in the same medium (Culture C) and an inoculation was also made from it into Malachite Green broth (Culture D). In all cases these cultures were re-inoculated into fresh tubes once a week and were all kept at 37° C.

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* Indicates *acid* reaction, but only very small bubble of gas.

Culture C.

This was plated out several times at intervals of about two months, but only one type of organism was obtained in every case. This type coagulated milk usually in 48 hours, produced only A., sl. G. in lactose, A., sl. G. to +reaction in dulcitol (in 8 to 12 days), + +reaction in glucose and A., sl. G. to +reaction in mannitol (in 48 hours). In salicin, acid usually appeared about the 7th day. This variety was quite permanent. Every endeavour was made to restore the original activity of the organism, without success.

It does not differ markedly from the original culture, but tested side by side the difference is decided.

Culture D.

This was the inoculation of Culture C made into Malachite Green broth (0·05 per cent.), as stated. It underwent no greater physiological changes than did C, milk did not coagulate until about the 15th day in most cases, and in lactose never more than the A., sl. G. reaction was obtained. Physiologically the organism was the same as Culture C and was as permanent; culturally it showed marked differences. Its power to grow at 20° C. was greatly inhibited. No growth was apparent until six days had elapsed, and then a very watery viscid streak appeared. Growth at 37° C. was quite normal.

Culture B.

This organism, on the other hand, became profoundly modified. After several re-inoculations in B.G. broth it was plated out and the colonies obtained on testing in the usual media gave: Milk, acid 48 hours, coagulated in about five days; lactose, glucose, and mannitol, acid in 48 hours, but no gas at any time; dulcitol, not attacked at all; salicin, sometimes acid after several days and sometimes not attacked. Every attempt to restore the original activity failed, the above features being quite permanent. One colony of this type was then started in Malachite Green broth (0·05 per cent.), in which it soon grew well.

After several re-inoculations, the colonies, on plating out on agar (+I) at 20° C., came up very slowly and were watery and very large ($\frac{1}{2}$-inch diameter). On testing, acid production was delayed in lactose and mannitol till the 5th day, but glucose was rendered acid in 48 hours. Sub-culture on agar at 37° C. restored acid production in lactose to 48 hours, but not in mannitol, which was still delayed. Gelatin sub-cultures at 20° C. were rather weaker physiologically than the original culture from which they were taken.

It must be carefully noted that only changes in physiological activity had occurred. The ordinary growth of all the organisms described above was quite normal and strong at 37° C. and was quite strong at 20° C., there being only a great tendency to delay in development. In every case before testing in the various peptone waters a strong growth was first obtained and heavy inoculations were used, and there was no trace of the dye stuff added to the test media with the culture. The effect which was produced in each case described above had been brought about by some impression made on the protoplasm as it was transmitted unchanged through successive cultivations on ordinary media.

The point which stands out clearly from the above results is that, from an original typical culture of *B. coli* obtained from a single cell, two strains have arisen, (1) a strain slightly modified by the dye stuff, but in a permanent manner *and refusing to be further affected*; (2) a strain gradually undergoing profound and increasing change in the same environment and resulting in an organism entirely different from the original culture, the strain being also of a permanent character.

It is important to notice that from one original organism there have arisen, by a simple process of cell division, at least two organisms, one of which is practically resistant to its environment, while the other has become greatly and progressively modified. It has been held that all such individuals should behave alike under similar circumstances, but it has been my constant experience that this is not the case. Failure to recognise this has no doubt led to the impression that organisms do not show variation.

Further, granting that these fermentative changes are brought about by enzymes present in the bacterial cell, it is evident that these are not an intrinsic and integral part of the protoplasmic substance. They may be entirely lost or greatly modified in activity, and, supposing that two enzymes at least are necessary to bring about the complete fermentation of the test substance, it is also evident that those which bring about the acid change may subsist while those which produce gas, etc., are completely lost.

Under these circumstances, it is not too much to suppose that in the life of the organism itself, the opposite phase may occur, and that, as under certain circumstances fermentative power is lost, so also, under some other set of circumstances, it may be acquired when it does not already exist.

Further Researches on the Extrusion of Granules by Trypanosomes and on their Further Development.

By W. B. Fry, Major R.A.M.C., and H. S. Ranken, M.B. (Glasg.), M.R.C.P. (Lond.), Captain R.A.M.C. (With a Note on Methods by H. G. Plimmer, F.R.S.)

(Communicated by H. G. Plimmer, F.R.S. Received December 23, 1912,—Read February 6, 1913.)

[Plates 9–11.]

Introduction.

In March, 1911, in the course of some work on trypanosomes carried out at the Wellcome Tropical Research Laboratories, Khartoum, the extrusion of certain granules from trypanosomes was observed by one of us (W. B. F.). The Director of the Laboratories, Dr. Andrew Balfour, was informed of these observations, and he himself shortly after observed a somewhat similar extrusion of granules from Spirochætes (spirochætosis of fowls), an account of which he published.*

In June, 1911, a preliminary note on the subject was communicated to the Royal Society by one of us (W. B. F.). Since then, a great deal of work has been done on the subject by us conjointly, but for the most part independently; by one of us (W. B. F.) at Khartoum and in London, by the other (H. S. R.) at Yei in the Lado Enclave.

As will be seen, the results recorded in the course of this paper go far to confirm the conclusions arrived at in the preliminary note, *i.e.* that the phenomenon is one connected with a stage in the life-history of the parasite, especially in chronic trypanosomiasis, in which it is found that the trypanosomes disappear from the blood of an affected animal for considerable periods.

These observations offer, too, an explanation of the infectivity of fluids, blood for example, which, while showing absolutely no trace of trypanosomes, will infect susceptible animals, a fact that all workers on trypanosomiasis are acquainted with; they also throw light on that condition which has been spoken of as "a possible ultra-microscopic stage" in these diseases.

Methods.

In the earlier part of these investigations two methods were principally used, they were:—

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* 'Brit. Med. Journ.,' April 1, 1911.

(1) Dark-ground illumination used in the ordinary way, but with the addition of a practically monochromatic light, which improved the definition.

(2) A method of "vital staining," the stain used being 0·75-per-cent. toluidin blue in physiological salt solution. This was mixed with blood, gland juice or other fluid to be examined, in a capillary pipette, blown on to a slide, covered with a cover-slip, and ringed with vaseline. With solid organs an emulsion in salt solution was used. The proportion of stain varied with the material from 1–3 to 1–8, according to the rate at which it was felt desirable to cause the staining to take place.

New methods of fixation and staining have also been used; these form the subject of a note appended to this paper. Both of these processes give practically the same results.

I. *On Granules in General in Trypanosomes.*

Besides the nucleus and blepharoplast there are other bodies in many trypanosomes which may, in the ordinary acceptation of the term, be called "granules."

There are certainly two classes of granules to be seen in trypanosomes: (1) those with which we are concerned—probably of nuclear origin and of infective nature; and (2) others which probably represent stored food material. The latter are of importance for us only because of the possibility of their being confused with the former, and here it may be stated that it has been found possible to fix and stain preparations so as to show a difference between them in staining reaction. Further evidence in favour of this differentiation was met with as a side issue in the course of experiments with hypertonic and hypotonic salt solutions, to be described later. It was found that when trypanosomes swelled up under the influence of these solutions many granules disappeared, leaving evident only from one to three. The inference seems to be that the granules which disappeared, owing to alteration in osmotic conditions, are of a quite different nature.

In this paper the word "granule" connotes those first mentioned, whilst the food granules are ignored in our descriptions, unless specifically mentioned.

The following varieties of trypanosomes have been available for study, and granules have been observed in all:—

(1) *T. gambiense* (Sudan), (2) *T. rhodesiense*, (3) *T. brucei*, (4) *T. evansi* (Sudan), (5) *T. nanum* (Sudan), (6) *T. pecaudi* (Sudan), (7) *T. lewisi*.

In all cases the granule, as seen by dark-ground illumination, is a small, sharply defined, highly refractile body, and on vital staining it takes up the toluidin blue rapidly, and shows as a deeply stained, more or less circular body, which contrasts with the lighter tint of the trypanosome body.

The number of granules apparently varies in different species of trypanosomes. They may also vary in size and number in the same species, *e.g.* a strain of *T. nanum*, obtained from cattle, was carried on by passage through gerbils. For two and a half months many of the trypanosomes contained a single large granule. At the end of this period the granule became multiple, and three or four could be seen; at the same time there was a great diminution in their size. It was noted that, coincidently with this increase in number of granules, the virulence of the strain became greater. We have found that granules are not necessarily always present in trypanosomes. At present we can only generally indicate the stage at which granules may develop,, and are unable to say what conditions determine their appearance; but the following details are the result of our observations:—

T. brucei was investigated in gerbils, in which the disease was fatal in six weeks, and during its course they showed at least two or three exacerbations with a large number of trypanosomes in the blood, with corresponding latent periods when they were absent.

When trypanosomes first appeared in the blood, whether at the beginning of the infection or after a latent period, it was observed that they did not contain granules; the latter developed about the fourth day after trypanosomes were first seen, and increased in size and number. For about 24 hours trypanosomes with granules were numerous. After this period, when free granules were numerous in the blood, the proportion of trypanosomes containing granules steadily diminished, till finally, though an enormous number of trypanosomes might be present, granules could not be found in any of them. This condition usually preceded a latent phase, or the death of the animal.

We have thus a definite sequence of events during an exacerbation of the disease:—

(1) Trypanosomes without granules.

(2) Trypanosomes showing granules which gradually become larger and very evident.

(3) Many free granules.

(4) Many trypanosomes but no contained granules.

(5) Trypanolytic crisis, or death of the animal.

This was also found to hold good with *T. nanum* and *T. evansi* (Sudan).

In the case of a goat inoculated with *T. brucei*, which lived for 133 days, and whose blood from the end of the first fortnight was always infective, no trypanosomes were at any time discoverable in the blood, which was examined daily for the first two months of the illness. In all the specimens of blood examined granules have been found. Similarly in the case of guinea-pigs and rabbits the blood has been found to be uniformly infective during the so-called latent periods, when no trypanosomes can be found in the blood by microscopic examination.

II. *Extrusion of Granule.*

The original observations have been repeatedly verified during the past 18 months, and we have been able to satisfy ourselves completely that extrusion of granules is a constant feature of trypanosomal infections.

The phenomenon has been observed in all species of trypanosomes studied with the exception of *T. lewisi.* We were able to assure ourselves of the presence of granules in that trypanosome, but the movements are so active that definite extrusion was never witnessed by either of us. On account of the high degree of motility the species was unsuitable for work on this subject, and prolonged observations were not made. The mechanism of extrusion has been studied in detail in *T. nanum* and *T. gambiense.*

(1) *T. nanum.*—The strain was obtained from infected cattle from the White Nile district, and, for the purpose of these observations, was kept up by passage through gerbils. This type of trypanosome is very convenient for the study of this process, as the granule is large and very evident and the trypanosome, whilst evincing active lashing movements, does not progress across the field of the microscope, but remains more or less stationary, so that there is no difficulty in watching the same trypanosome through all the phases over a period of several hours, if necessary. Further, an animal can be selected at a period when extrusion is a frequent occurrence.

When extrusion is about to take place the granule begins to work its way slowly, but quite distinctly, from the centre of the trypanosome towards the pointed extremity. Arrived there, it makes its way back to the centre. This takes place quite often—as many as seven or eight such movements having been observed. During these passages the granule can be seen distinctly bulging the periplast as if becoming more and more superficial—this bulging being strikingly apparent at the pointed extremity. Probably this movement is largely due to the movements of the trypanosome itself. Finally, the granule, stretching the periplast to a greater extent, is extruded suddenly from the pointed extremity and becomes a free element in the surrounding medium. Plate 9, fig. 1, illustrates all these stages.

(2) *T. gambiense.*—Here the preparations were made direct from cases of human trypanosomiasis.

In this species the granules are multiple and move rapidly backwards and forwards in the long axis of the trypanosome. They exhibit also a dancing movement and appear to throw themselves against the periplast and rebound from it. Sometimes the granules approach the surface, and in so doing may actually cause a slight protrusion on the covering membrane. This seems to be preliminary to extrusion, as afterwards the granule may be shot out with a certain degree of force into the free fluid to some distance from the host. In this species extrusion is not as a rule effected from the extremity, but from some point near the middle of the trypanosome body.

In infections running a very rapid course—such as *T. brucei* and *T. rhodesiense* in white rats—extrusion is readily observed, whereas in sleeping sickness in man, a very chronic infection, prolonged search may be necessary. Certain intermediate types of the disease are particularly suitable for study of this subject—for instance, *T. brucei* in gerbils, as described above. In the course of this infection granules are not extruded when the trypanosomes first appear. At a later stage the phenomenon is easily seen, and again it cannot be observed just before disappearance of trypanosomes from the blood. These facts tend to confirm our opinion that extrusion occurs at a definite period in the life of an adult trypanosome.

Extrusion can be stimulated by the administration of drugs and by certain mechanical effects such as variations in osmotic conditions. Reference is made to extrusion induced by varying strengths of salt solution in a later section of this paper.

Under ordinary circumstances extrusion of granules does not appear to have a prejudicial effect on the trypanosome. In warm wet preparations it can be seen to continue its movements and it apparently lives as long as the others. On the other hand, it has been shown above that extrusion of granules, if occurring generally, apparently heralds a disappearance of trypanosomes from the blood and is, in fact, the precursor of a trypanolytic crisis. Under unfavourable circumstances, *e.g.* after treatment, extrusion is followed by rapid disintegration of the trypanosomes.

III. *Effect of Drug Treatment on Extrusion.*

Certain phenomena in connection with the liberation of granules have been observed after treatment with antimony. Cases of sleeping sickness were given an intravenous injection of metallic antimony, and gland-puncture wet preparations made at short intervals after treatment, 3 minutes, 5 minutes, and so on. These were examined by dark-ground illumination and the results of the observations are here described.

Extrusion of granules is more frequent. The exaggerated motility is one factor, and the protoplasm, and more particularly the periplast, seem to lose elasticity, with the result that the granules can get free more easily. If a granule is forcibly ejected by energetic movements of the trypanosome it is flung out into the free fluid to some distance; this is the most usual method.

Some trypanosomes seem to be acutely poisoned by the antimony, and death and complete dissolution occur very rapidly. This is more frequently seen when the preparation is made 5 to 7 minutes after injection of antimony. The trypanosome becomes anchored, its lashing movement slows down and comes to a standstill, the body swells and becomes bloated, losing its characteristic form. In this condition it is devoid of energy and can no longer forcibly extrude granules, but the latter have not suffered so severely and may still show an excited dancing movement inside the degenerate trypanosome body, which appears to give way before this activity, and the granule may ultimately work its way clear of the degenerate protoplasm and inelastic covering of the now dead, or dying, trypanosome.

In other instances the trypanosome does not die so rapidly and the granules, after continuing this dancing movement inside it for some time, gradually come to rest before the trypanosome has reached so advanced a state of degeneration as to permit a dancing granule to escape by its own efforts. The degeneration of the trypanosome continues till it has lost outline and refractility and can only be recognised as an ill-defined "ghost," enveloping the granules which are held in position—more or less in the original long axis of the trypanosome—by this viscid protoplasm. This is the last stage that can be seen in a dark-ground preparation where the objects are at rest. In the living subject, however, it is probable that this degenerate protoplasm would not be allowed to remain at rest, but would be broken up by the active currents and eddies and the granules would thus be set free.

Thus there are probably three methods by which a granule may be liberated from the parent trypanosome:—

(1) By the activity of the trypanosome—forcible extrusion.

(2) By the active movement of the granule in a rapidly degenerating trypanosome.

(3) By outside agencies, eddies, currents, etc., which may break up a degenerate trypanosome when the contained granules are unable to effect their escape.

In some cases extrusion occurs rapidly. A trypanosome has been seen to extrude two large granules and immediately afterwards break up—the whole process being complete in 20 minutes.

In the early preparations (3 minutes) the exaggerated motility is a prominent feature and forcible extrusion is most commonly seen; in the later films (7 minutes) the antimony has had longer time to act and the phase of hyperactivity has passed. It is then more usual to see the more gradual escape of the granule, and as the trypanosomes are "anchored" they can be kept under observation more easily. On several occasions where death has occurred slowly we have been able to watch a trypanosome for periods up to four hours. In the 20-minute preparations trypanosomes have never been found, but granules are very numerous. The activity of the freshly extruded granules after antimony is much greater than the movements of granules seen before treatment.

IV. *The Free Granule.*

The granule free in the blood or fluids is seen to be a small spherical or pear-shaped body. In dark-ground preparations it is seen to be highly refractile, and by its activity it causes considerable disturbance in the surrounding fluid; with vital staining this young granule takes on the stain rapidly and uniformly, and seems to be undifferentiated. It frequently remains near its former host for some little time before showing independent movement. At first only a dancing movement may be seen; this, however, is a preliminary phase, and soon the granule begins to move slowly across the field, turning over on itself. There is no doubt as to the motility: they have often been observed to move out of a microscope field in preparations where there was no question of currents, etc. In our opinion a pseudopodial protrusion appears early, which at first is short and rather thick.

In animal infections and in cases of sleeping sickness in man, granules are found in the blood, glands, and internal organs. They are, of course, much more numerous in animals in which the adult parasites appear in great numbers. In experimental animals granules have been found in the proximal glands 24 hours after inoculation. This fact seems to be of great importance.

The criterion in the recognition of granules must be motility,* but their greater affinity for such stains as toluidin blue is of undoubted assistance in distinguishing them from the countless small bodies seen in wet preparations, *e.g.* blood-platelets and leucocyte granules.

V. *Further Development of Granule.*

So far we have shown that the trypanosome discharges living elements endowed with motility, and showing the same reaction as nuclear material to toluidin blue. The further stages are more difficult to follow, as all stages cannot be seen in any individual preparation. We have endeavoured, so far as possible, to correlate the various appearances met with; at the same time we cannot be sure that we set them out in exact chronological order.

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* The addition of a small quantity of cherry-gum solution to the preparation will differentiate between Brownian and vital movement. It stops the former and slows the latter.—H. G. PLIMMER.

In the first place granules, like many other free bodies in the blood plasma, are liable to undergo phagocytosis, and have been seen in all conditions within polynuclear leucocytes.

Granules somewhat older have also been seen in hyaline mononuclear and in endothelial cells, but in cells of this type, on the other hand, the contained granules are quite unchanged, and we are unable to say that they are being destroyed. It is possible that they may be entering on an intracellular phase of existence. They have been very well seen by one of us (W. B. F.) in a large mononuclear leucocyte during examination of the blood of a cat infected with *T. nanum*. They have also been seen in endothelial cells in liver puncture preparations from cases of sleeping sickness.

The first change seen in the free granule is a slight enlargement and elongation, rendering it more definitely pear-shaped. Then one begins to note a slight differentiation of structure into a central area staining a dark blue or purple, and a peripheral zone which is only faintly tinted blue. The enlargement is progressive, and the body becomes more uniformly blue, while a small dark blue or purple spot is visible, varying in position from the centre of the body to the apex. This may be assumed to be the earliest differentiation of cytoplasm from nuclear material. At this stage there is sometimes a definite flagellum-like projection which is usually short and rather thick, and more like a pseudopodium (Plate 11, fig. 2).

The same early forms have been studied in dark-ground preparations from bone-marrow in animals infected with *T. nanum* and are illustrated in Plate 9, fig. 2, A to I.

From this point the body enlarges, and the flagellum-like body becomes relatively, if not actually, reduced in size, so that forms are seen as in Plate 11, figs. 3 and 4. Later on the mass of chromatic material divides, and two are seen—one much smaller than the other. The body then becomes more rounded. Some are regularly spherical, while others show projections from various points, and have on surface view a roughly triangular appearance.

At this time of their development they resemble very closely the Leishman-Donovan bodies in Kala-azar; they are found sometimes in enormous numbers in lungs, bone-marrow and spleen. Death in acute trypanosomiasis is caused by plugging of the cerebral capillaries with these forms. This cause of death is very similar to that in pernicious malaria.

From this stage—the binucleate body—there appear to be two directions in which the further development may proceed. The body may enlarge slightly, develop a true flagellum from the neighbourhood of the micronucleus, and then become longer. This increase in length continues, and the macro- and micro-nucleus in this process become further separated; the flagellum comes to lie along the margin, and this form can now be recognised as an early immature trypanosome. There is no undulating membrane, but development proceeds till the adult form is reached.

On the other hand, the circular form may enlarge to a greater degree, and show a larger amount of a pale-blue staining cytoplasm that seems characteristic of young forms. The nucleus and micronucleus then undergo division by schizogony, but remain within the single mass of cytoplasm. The time of appearance of the flagellum seems to be variable, but ultimately all the pairs of macro- or micro-nuclei come to have a flagellum with a fan-shaped origin usually projecting beyond the margin of the cytoplasm.

Plate 11 shows forms with two, four, and eight macro- and micro-nuclei and flagella. We have seen indications of similar forms in vital preparations, but the latter cannot show the same detail as fixed and stained preparations. We have no knowledge as to the conditions which determine either of these events—possibly in the latter case there may be some sexual process either in the cells or fluids.

Many of these bodies are identical with the Plimmer and Bradford bodies, which they described in 1902,* and we have found them in preparations made from many different animals and from man, of glands, internal organs and bone-marrow. They show when living undoubted motility, but the early granule shows much more active movements than these later forms. The fact of their showing this vital property, however, precludes any possibility of their being degeneration forms.

In a few cases of sleeping sickness in man some other bodies have been seen by the vital method in fluid obtained by liver puncture. In the majority of instances some blood was mixed with the liver juice; this diluted the fluid and the bodies were very scanty, but the appearances presented suggested that some process of division was going on. Protoplasmic masses were seen containing four or eight small ovoid bodies taking on nuclear stain, but there was no nuclear differentiation. These were seen only in wet preparations, and could not be preserved.

Another form was seen as a fusiform body lying round a segment of the periphery, apparently of a mononuclear cell. It suggested an immature trypanosome, and this idea was confirmed by the presence of similar bodies free in the liver juice showing slight sluggish movement.

* 'Quart. Journ. Micros. Sci.,' February, 1902.

VI. *Fixed and Stained Specimens.*

The foregoing sections have dealt with living trypanosomes, but we were not able by the ordinary methods to make permanent preparations showing the various stages and forms, and demonstrating the staining reactions of the granule from its origin as a nuclear bud onwards.

Mr. H. G. Plimmer, F.R.S., has appended a note describing special fixing and staining methods devised by him, and we wish to state that it is only by the use of these methods that we have been able to confirm the appearances we have described in unfixed wet preparations, together with the differentiation between vital and nutritive granules.

In regard to the granule within the trypanosome, films have been stained showing the granule taking origin from the macro-nucleus itself as a small bud with characteristic chromatin reaction. All the stages of separation have been seen till the granule is a small, independent, dense, chromatin-staining mass in the cytoplasm (Plate 10, figs. 1–6). The granules, as stated, vary in number, and are most frequently seen between the macro- and micronucleus. They stain a deep red and show a remarkable contrast to the food granules which have taken on the iodine reaction from the fixation, and are visible as bluish-staining bodies or sometimes as a fused mass. This can be better seen in certain bird trypanosomes on account of their large size.

In a certain number—probably the larger number—of instances the granule at some stage of its development is surrounded by a faint-staining hyaline circular or ovoid area. It is probable that in such cases the granule is really within a vacuole (Plate 10, fig. 8). Sometimes the granule appears to be spherical, but in other cases, even when being budded off from the nucleus, it already shows as an elongated, pear-shaped body; this is well seen in Plate 10, figs. 2–4.

Granules can be seen actually causing a protuberance on the periplast and evidently on the point of being extruded. Others have been fixed when half-way out, while free granules, which have just effected their escape, have been seen lying close to the parent trypanosome. The early free granule takes on the chromatin stain deeply, and is identical with the body observed by the vital method.

The observations as to phagocytosis have been confirmed and more advanced forms, showing a macronucleus, micronucleus, and flagellum, have also been seen within polynuclear cells, and rounded forms resembling the Leishman-Donovan body have been demonstrated in large mononuclear cells.

All stages have been seen from the early free granule; the protoplasm becomes more visible, and increases in amount; the nuclear material becomes differentiated from it and more concentrated, and then we are able to see early forms with a macronucleus and micronucleus. The macronucleus in the circular forms may be spherical or may become elongated and spread out along the periphery. Some forms show much more protoplasm: it stains a pale blue and sometimes shows some faint pink granules. The flagellum varies in length, but is relatively much longer than that of the adult trypanosome. In the internal organs, and especially in the lung, there may be enormous numbers of these small rounded bodies with macro- and micronuclei, with or without flagella, sometimes separate and sometimes massed together.

A further stage has been observed in these masses; they have been seen just on the point of disruption, some of the small bodies were separating, and lay at varying distances from the main mass. Each showed the two nuclear elements with a small body of homogeneous cytoplasm.

In addition, forms such as mentioned on p. 385 have been seen—large masses of protoplasm with two, four, or eight macronuclei, and corresponding micronuclei, which are, as a rule, placed close to the macronuclei and stain very densely. The flagellum can be seen arising from a line equal in length and close to the micronucleus, in a fan-shaped collection of very fine filaments which unite to form a flagellum (Plate 11).

In smears of blood or organs advanced single forms—*i.e.* with one macronucleus, micronucleus and flagellum, and a relatively large amount of protoplasm—can be seen, and all stages from this to the adult trypanosome (Plate 11). A series has been prepared showing an almost imperceptible gradation from the granule stage up to adult trypanosomes.

Up to this point we have only referred to the work of Bradford and Plimmer in their paper on *Trypanosoma brucei* and its development. In this paper and in the plates they have described and figured the granules within the trypanosomes, the free early bodies, the more advanced single forms called "amœboid" and the disrupted schizogonous bodies called "plasmodial masses."

Our work was carried out at a time when we had no access to the paper, and this makes it all the more remarkable that the forms we describe should so closely resemble, and indeed confirm, many of the appearances described in 1902, and we feel that in many respects we can add little to the original work, beyond demonstrating the vital properties of these bodies.

We should like to draw attention to the fact that early granules, forms with short flagella and small round forms, are figured by Mott* in his

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* 'Reports of the Sleeping Sickness Commission of the Royal Society,' No. VII December, 1906.

"Histological Observations on Sleeping Sickness and other Trypanosome Infections."

VII. *Some Animal Experimental Work in Reference to Granules.*

A number of experiments were undertaken to ascertain if it were possible to infect animals by granules alone. To do this, fluid containing granules and no trypanosomes was required. It was thought possible that the granules (if reproductive elements) might prove more resistant to changes in their environment than adult trypanosomes. In order to test this, blood showing a heavy infection was added to a hypertonic salt solution, up to 2 per cent.

It was found on mixing one volume of infected blood with two to three of salt solution and keeping it at temperatures between 34° and 38° C., that after standing for 5 to 10 minutes individual trypanosomes began to swell up and become globular and the contained granule or granules to become active, moving about in the now spherical trypanosomes; after a short period the granules escaped from the containing membrane and became free. The remnant of the trypanosome was left as a faintly discernible spherical body with no characteristic features.

This process of escape of granules continued until no formed trypanosomes could be found; at the end of from half to three-quarters of an hour the process, as a rule, appeared complete. There are apparently several factors which influence the occurrence of this phenomenon, the temperature, the hypertonicity of the solution, the stage of development of the trypanosomes, and the strain worked with.

If, whilst looking at one of these slides during the process, an individual trypanosome be watched, it will be noticed that its active movements suddenly become slowed, and then, as though blown steadily out by some entering fluid, the trypanosome, in the course of about 3 to 10 secs., is changed from its usual shape to that of a round body in which the granule or granules are freely motile. The escape of the granule takes place, as a rule, a few minutes after this.

Infection was obtained repeatedly, and the following are details of two positive results:—

No. I.

November 5.—Gerbil (F. 10) injected with about 0·2 c.c. of treated blood obtained from a gerbil infected with *T. nanum* (heavy infection). The injected blood was treated with sodium chloride solution 2 per cent. and sodium citrate 1 per cent. for one hour. At the moment of injection no living trypanosomes could be distinguished; sphere forms and free granules very numerous.

November 10.—Trypanosomes first found in blood.

November 11.—Trypanosomes very numerous.

November 14.—Gerbil found dead; spleen very large.

No. II.

November 5.—Gerbil (F. 11) injected with blood (0·2 c.c.) obtained as above, but after two hours' standing no trypanosome could be seen, only round forms and free granules.

November 12.—Trypanosomes first found in blood.

November 14.—Trypanosomes very numerous.

November 15.—Gerbil found dead; spleen very large.

The average time of infection in gerbils is four to six days after ordinary inoculation. Similar results were also obtained with dogs.

These experiments are, of course, not absolutely conclusive, but so far as could be ascertained microscopically the granules were the only discernible remnants of the trypanosomes which retained their characteristic form.

Further experiments were also made to trace if possible the fate of granules so injected into animals. Inoculations were made with solutions containing large number of free granules, and the animals were killed before trypanosomes could be found in the blood. Granules and the later forms in various stages of development were found in the proximal glands, also in the internal organs.

Note on a New Method of Blood Fixation.

By H. G. Plimmer, F.R.S.

During some years of work on the blood of animals, many methods of fixation have been tried, principally with the view of obtaining a better fixation of blood parasites. The method described below has fulfilled this object better than any other, and is more faithful than even osmic acid.

The use of iodine for the fixation of unicellular organisms dates from the work of Kent in 1881 on the Infusoria, but the application of it to blood is, so far as I know, new.

I have used iodine in two forms, in vapour and in solution, and each has its special advantages. When a blood-film is exposed wet to the vapour from a solution of iodine in chloroform, the fixation of the various elements is practically instantaneous, as the penetrative power of iodine in this form is greater than that of any other fixative known to me; there is less alteration both in form and size of the cellular elements and parasites than with any other fixative. When used in solution several things happen which are of value in enabling very fine structures to be more easily made out.

If blood be mixed with a solution of iodine in salt solution containing iodide of potassium, certain elements and parasites, especially trypanosomes, swell up so that the finer parts of their structure, for instance the nucleus and blepharoplast, are much clearer and more definite than with the ordinary methods. The nucleus shows as clearly as, if not clearer than, when Flemming's solution and iron-hæmatoxylin have been used. There is the clear space containing the karyosome, and surrounding this, in many cases, are seen a number of granules, some of which can be seen budding off. The blepharoplast is clearly seen as a structure quite distinct from the micronucleus, and the earlier stages of division of a trypanosome, *i.e.* the division of the blepharoplast and the formation of a second undulating membrane extending down the body of the trypanosome and forming eventually a second flagellum, can be seen and followed easier than with any other mode of fixation. For the smaller forms found in spleen, glands, and marrow of animals with chronic trypanosomiasis, this method, by causing swelling of the elements, renders the very small forms distinct, and renders their nuclear structures much more visible.

Both these methods are also the best I have found for avian and reptilian blood containing parasites, *e.g.* filaria, malaria, hæmogregarines, etc.

The steps of the two methods are here detailed. Either slides or cover-glasses can be used, but in all blood-work the best results are obtained with cover-glasses. After the Giemsa or fuchsin staining the definition is greatly increased by the use of a green monochromatic screen, such as Wratten's No. 19, which shows the picture in blacks and greys.

I. *Vapour Method.*

1. Expose the thinnest possible film whilst wet to the vapour of a solution of iodine in chloroform for 10–15 seconds until it is distinctly yellowish.

A hollowed glass block does for cover-glasses, and a glass cylinder of suitable height, with the iodine and chloroform in a small vessel at the bottom, does for the slides. In cold places the vessel should be warmed in order to get the vapour given off freely.

2. Place the film when it has become just surface dry (a dead, mat surface, not really dry) in chloroform, or in alcohol and ether, equal parts, for two hours. I use chloroform for cover-glasses and alcohol-ether for the rougher slides.

3. There will now be no free iodine left in the film, and it can be stained in many ways. I use the following :—

A. *a.* Drop 3–8 drops of Giemsa's solution on the film, and immediately after double the number of drops of distilled water. Leave for from 2 to 12 hours.
  b. Wash well with tap-water.
  c. Drop on 2–8 drops of orange-tannin solution and leave for 15 seconds.
  d. Wash thoroughly with tap-water, up to two minutes.
  e. Dry with filter-paper.
  f. Mount in cedar oil or liquid paraffin.

B. *a.* Carbol-fuchsin for from 2 to 12 hours.
  b. Wash in tap-water.
  c. Alcohol until free from bulk of stain.
  d. Differentiate in clove oil saturated with orange G.
  e. Stop when desired by washing in xylol.
  f. Mount in cedar oil or liquid paraffin.

C. Iron-hæmatoxylin may be used in any of the ordinary ways. Kernschwarz for 24 hours gives very delicate results.

II. *Solution Method.*

1. Make a saturated solution of potassium iodide in 0·8-per-cent. salt solution and add iodine to saturation.

2. Mix 5–6 drops of this with 10 c.c. of salt solution.

3. Mix in a marked pipette equal parts of this and the blood to be examined. In the case of organs small pieces may be crushed in an equivalent quantity of the iodine solution to form an emulsion.

4. Take large drops and make a thickish film. Wait until the surface has begun to dry (as in I), and place in alcohol and ether for two hours.

5. Continue as under 3.

DESCRIPTION OF PLATES.

Plate 9.

Fig. 1.—Series to illustrate mechanism of extrusion of granules in *T. nanum* (see p. 380).

„ 2.—Developmental forms of *T. nanum*, seen in bone-marrow; the progressive tendency towards the characteristic shape of the adult trypanosome is shown. Dark-ground illumination, Leitz $\frac{1}{12}$ objective, N.A. 1.30, compensating eyepiece. × 8.

The earliest form, A, shows no evidence of a protoplasmic envelope and has the appearance of a well-developed granule just after extrusion. In B the cytoplasm is clearly evident and the separation of the micronucleus has commenced. C shows a well-developed form, of circular shape, with the nuclei shown at a distance from each other.

D, E, F, and G show the progressive increase of protoplasm, the last form being almost trypanosomal. H is a young trypanosome, and I an older one in which a flagellum is evident.

These forms were all living when drawn.

Plates 10 and 11.

All the figures are drawn under a Zeiss 3-mm. apochromatic objective, N.A. 1.40, with compensating ocular. × 12.

Plate 10.

Figs. 1–8.—*T. rhodesiense* in rat's blood, showing granules from their origin to extrusion.

Figs. 9–16.—From blood and liver of rat infected with *T. rhodesiense.*

Figs. 17–22, 24, and 26.—Are from the spleen of a guinea-pig infected with Nagana which lived three months, and showed no trypanosomes in the blood for some time before death.

Figs. 23 and 25.—From a lymphatic gland of a cat infected with Nagana.

Fig. 1.—Four granules are seen in the trypanosome-body, and another is in an early stage of being budded off from the macronucleus at the right upper angle.

„ 2.—Two granules are seen coming off the macronucleus. The one on the left is still attached and shows the elongated form.

Fig. 3.—A similar elongated granule is seen completely separated from the nucleus. There is a faint indication of a halo surrounding it.
,, 4.—A large elongated granule is seen between the macro- and micronuclei, lying close to the periplast.
,, 5.—Several granules are present ; one is just being detached from the macronucleus.
,, 6.—Two granules are seen on the point of escaping from the trypanosome ; the larger looks as if it is nearly extruded.
,, 7.—A recently extruded granule is seen near the trypanosome. The macronucleus shows two deeply stained points—probably granules becoming differentiated in its substance before being budded off.
,, 8.—Two granules, lying between the macro- and micronuclei, are each seen to be surrounded by a well-defined clear hyaline area. Two others are almost completely separated from the macronucleus.
,, 9.—Free granule ; no differentiation.
,, 10.—Free granule, larger, and with a faint rim of cytoplasm.
,, 11.—Ring-shaped nucleus with micronucleus coming off ; definitely more protoplasm than the previous form.
,, 12.—Early form with macro- and micronucleus and pale blue-staining cytoplasm.
,, 13.—Similar form, larger.
,, 14.—The nucleus has divided in this specimen, while there is only one micronucleus seen.
,, 15.—Both macro- and micronuclei are divided.
,, 16.—Micronuclei only have divided ; macronucleus in process of division.
,, 17–26.—All are similar forms. They vary in shape and correspond closely with the forms seen by vital staining of emulsions of internal organs.
,, 20 and 22.—Show division of the micronuclei.
,, 21 and 22.—Show the third chromatin body described.
,, 25.—Shows division of macro- and micronuclei.
,, 27.—A single form, with macro- and micronucleus, and a very long flagellum.

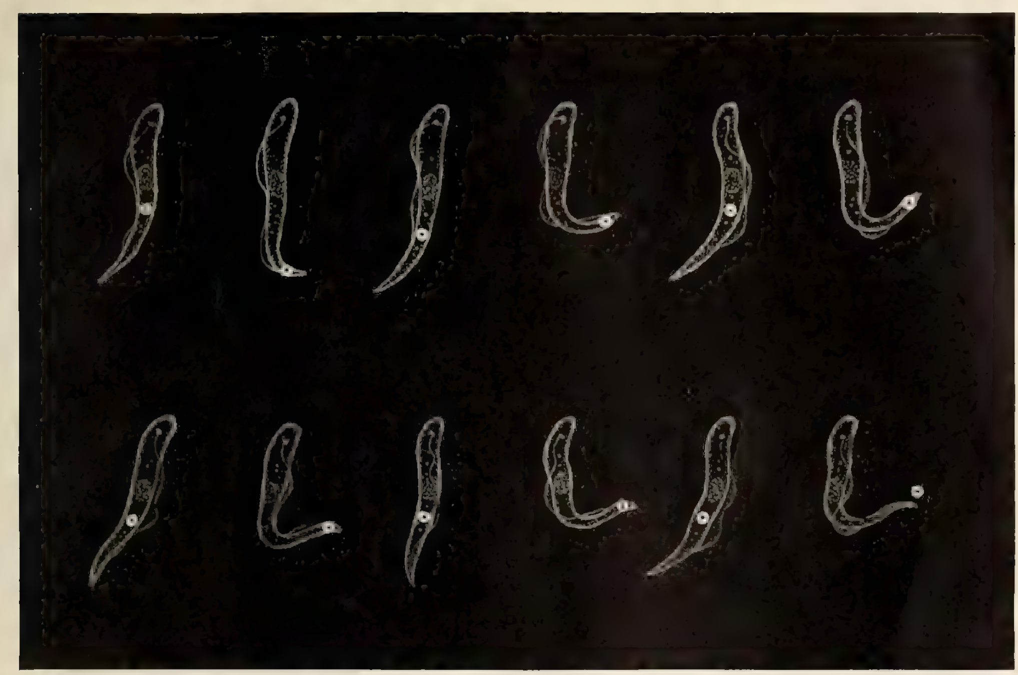
Plate 11.

Figs. 1–12.—The specimens were found in smear preparations from the liver and kidney from rats infected with *T. rhodesiense.* They show dividing forms in various stages.

Figs. 13–20.—Blood from liver of rat infected with *T. rhodesiense.* Immature trypanosomes are shown gradually merging into adult forms.

Fig. 1.—Early stage of division. There are already two micronuclei, but the macronucleus is just beginning to divide.
,, 2.—This shows similar division to fig. 1, but a little further advanced. The macronucleus is now in the stage of mitosis.
,, 3.—Complete separation of macro- and micronuclei, but the flagella are not yet separated.
,, 4.—Two form with nuclei and flagella completely divided ; one flagellum is much longer than the other and lies round the margin of the body.
,, 5.—Two form beginning to divide into two independent bodies which are identical with the early immature forms shown in figs. 13-15.
,, 6.—Two form. The nuclei have moved to some distance from each other. A thick fan is seen in the shorter of the two flagella.

Fig. 1.



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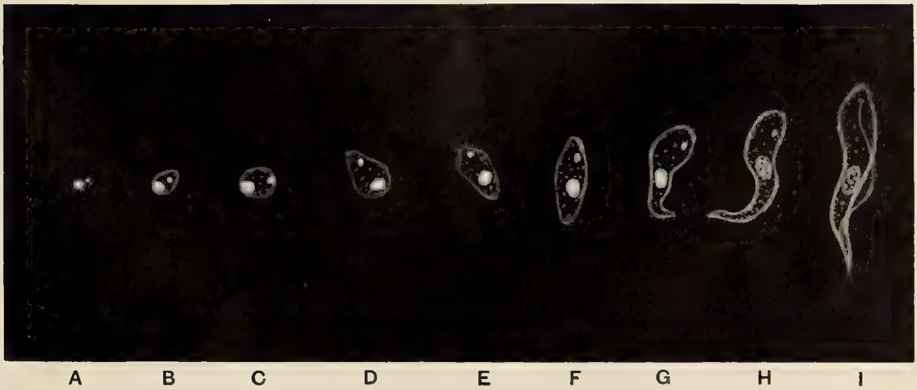

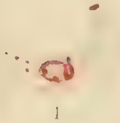
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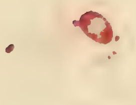
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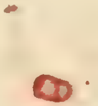

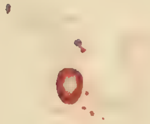
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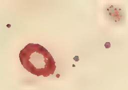
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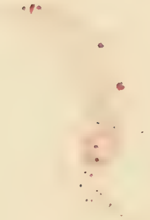
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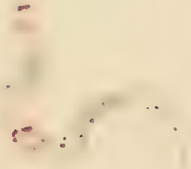
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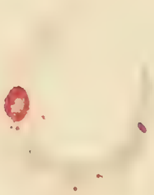
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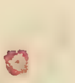
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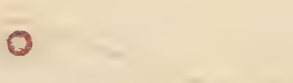
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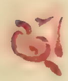
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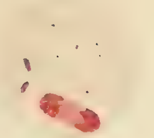
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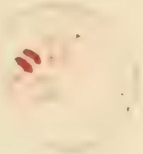
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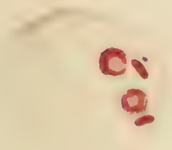
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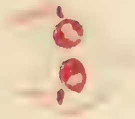
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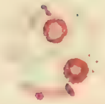
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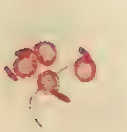
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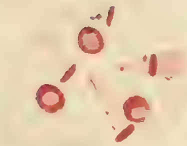
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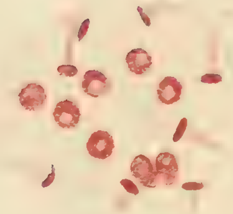
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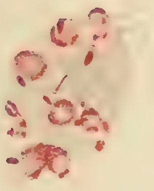
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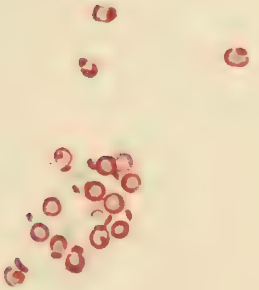
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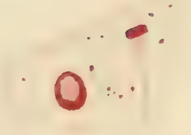
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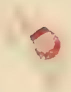
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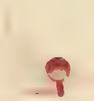
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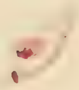
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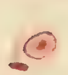
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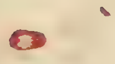
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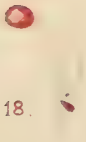
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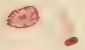
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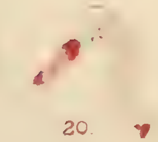
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Fig. 7.—Four form (early), the macronuclei have evidently recently divided. The two lower are moving away from each other; the upper have not completely separated.

" 8.—More advanced four form; all the pairs of macro- and micronuclei have moved away from each other.

" 9.—Eight form, a large body of cytoplasm whose margin shows a few indentations as if there might later be division of the whole mass at these situations. All the macro- and micronuclei and flagella can be seen.

" 10.—Eight form beginning apparently to divide; the cytoplasm shows lines of cleavage along the lower part of the outline.

" 11.—Mass of 16 bodies breaking up. These resemble the Leishman-Donovan body; each has a macro- and micronucleus, but no flagellum.

" 12.—A large form with single macronucleus and large micronucleus showing fan-shaped origin to flagellum.

" 13.—The body is rounded and has a clear blue-staining cytoplasm. The flagellum shows the fan-shaped origin well and stands straight out from the body. The micronucleus lies close to the macronucleus.

" 14 and 15.—The body is longer, and the flagellum is lying along the margin; the micronucleus is now moving away from the macronucleus.

" 16, 17, and 18.—These features are more marked, and the specimens show gradual approximation to adult type. The flagellum is seen to be separated at some point from the outline of the trypanosome body, the earliest stage in the development of an undulating membrane.

" 19.—The undulating membrane is now clearly present, but the trypanosome can still be recognised as immature by the fan-shaped origin of the flagellum and the pale homogeneous cytoplasm.

" 20.—An early adult trypanosome; the flagellum no longer shows the fan-shaped origin, and is much longer. Early granules can be seen in the cytoplasm.

Morphology of Various Strains of the Trypanosome causing Disease in Man in Nyasaland.—The Wild-game Strain.

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; Majors DAVID HARVEY and A. E. HAMERTON, D.S.O., R.A.M.C.; and Lady BRUCE, R.R.C.

(Scientific Commission of the Royal Society, Nyasaland, 1912.)

(Received February 24,—Read April 10, 1913.)

Introduction.

Trypanosomes of this species, isolated from five antelope, are here described and compared with the Human strains which formed the subject of a previous paper.* The Wild-game strains were isolated by injecting blood of antelope into susceptible animals. The blood was, as a rule, injected into a healthy goat, monkey, and dog, and from these other animals were inoculated.

Trypanosomes from the following species were studied: reedbuck, waterbuck, oribi, and hartebeeste. In these experiments, with the exception of the oribi, the three inoculated animals became infected. In the case of the oribi the blood was inoculated into a monkey and dog, no goat being available, and the monkey alone took the disease.

I. *Morphology of Strain I, Reedbuck.*

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest:—

Table I.—Measurements of the Length of the Trypanosome of Strain I, Reedbuck.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	21·7	34·0	16·0

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* *Supra*, p. 285.

Table II.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain I, Reedbuck.

	In microns.								
	16.	17.	18.	19.	20.	21.	22.	23.	24.
Total	5	30	57	90	81	53	27	17	16
Percentages	1·0	6·0	11·4	18·0	16·2	10·6	5·4	3·4	3·2

	In microns.									
	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.
Total	18	23	18	25	18	9	8	2	2	1
Percentages	3·6	4·6	3·6	5·0	3·6	1·8	1·6	0·4	0·4	0·2

Chart 1.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain I, Reedbuck, taken on nine consecutive days from Rat 847.

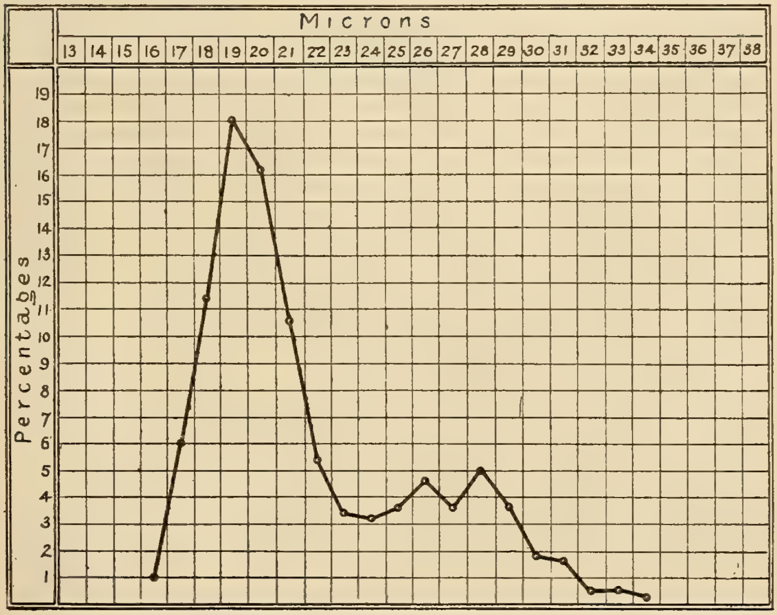


Table III.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain I, Reedbuck.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
July 17	847	Rat	4
" 20	847	"	2
" 22	847	"	2
" 23	847	"	4
" 24	847	"	9
" 25	847	"	10
" 26	847	"	19
" 27	847	"	22
" 29	847	"	4
		Average	8·4

II. *Morphology of Strain II, Waterbuck.*

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest:—

Table IV.—Measurements of the Length of the Trypanosome of Strain II, Waterbuck.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	23·5	33·0	16·0

Table V.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain II, Waterbuck.

	In microns.								
	16.	17.	18.	19.	20.	21.	22.	23.	24.
Total ……………	1	2	8	26	59	74	58	58	44
Percentages ……	0·2	0·4	1·6	5·2	11·8	14·8	11·6	11·6	8·8

	In microns.								
	25.	26.	27.	28.	29.	30.	31.	32.	33.
Total ……………	27	34	33	26	17	19	9	3	2
Percentages ……	5·4	6·8	6·6	5·2	3·4	3·8	1·8	0·6	0·4

CHART 2.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain II, Waterbuck, taken on nine consecutive days from Rat 1220.

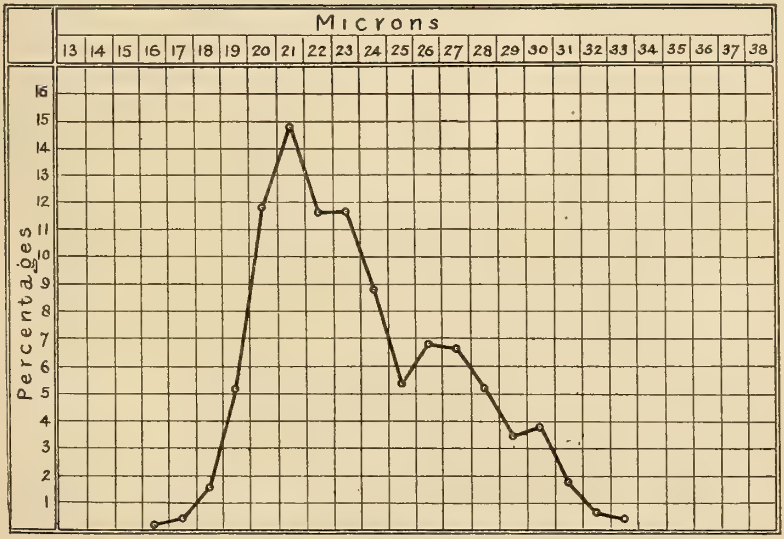


Table VI.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain II, Waterbuck.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
Sept. 7	1220	Rat	7
„ 8	1220	„	2
„ 12	1220	„	12
„ 16	1220	„	48
„ 17	1220	„	39
„ 18	1220	„	45
„ 19	1220	„	21
„ 20	1220	„	50
„ 23	1220	„	36
„ 24	1220	„	47
		Average	30·7

III. *Morphology of Strain III, Oribi.*

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest:—

Table VII.—Measurements of the Length of the Trypanosome of Strain III, Oribi.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	21·6	33·0	16·0

Table VIII.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain III, Oribi.

	In microns.								
	16.	17.	18.	19.	20.	21.	22.	23.	24.
Total	1	10	22	77	109	90	57	28	19
Percentages	0·2	2·0	4·4	15·4	21·8	18·0	11·4	5·6	3·8

	In microns.								
	25.	26.	27.	28.	29.	30.	31.	32.	33.
Total	23	15	21	14	6	5	2	—	1
Percentages	4·6	3·0	4·2	2·8	1·2	1·0	0·4	—	0·2

CHART 3.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain III, Oribi, taken on nine consecutive days from Rat 992.

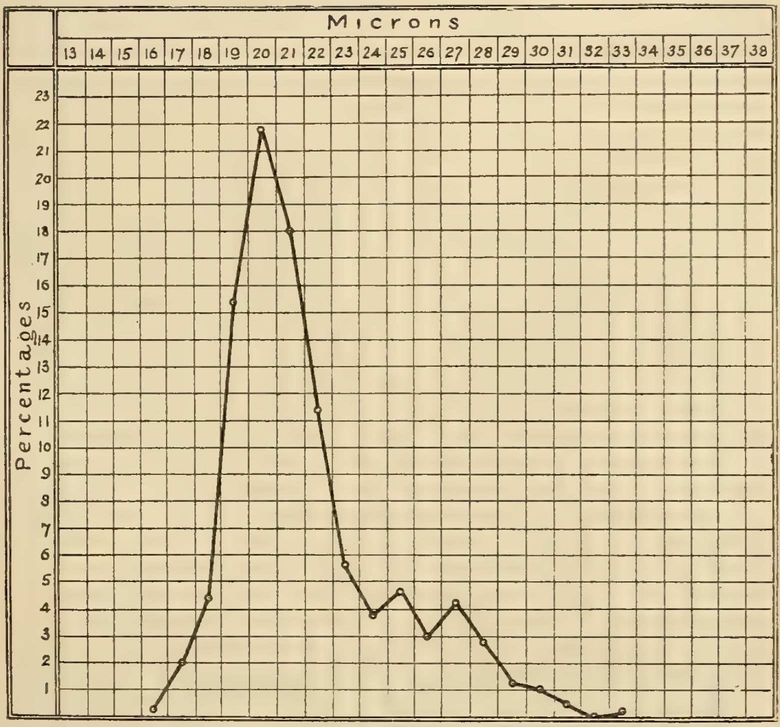


Table IX.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain III, Oribi.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
Aug. 1	992	Rat	12
„ 2	992	„	14
„ 3	992	„	10
„ 5	992	„	34
„ 6	992	„	15
„ 7	992	„	42
„ 9	992	„	29
„ 10	992	„	52
„ 12	992	„	42
„ 13	992	„	53
		Average	30·3

IV. *Morphology of Strain IV, Hartebeeste.*

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest:—

Table X.—Measurements of the Length of the Trypanosome of Strain IV, Hartebeeste.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	23·5	35·0	18·0

Table XI.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain IV, Hartebeeste.

	In microns.																	
	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.
Total	1	12	53	80	92	53	46	45	28	25	21	10	19	6	6	2	—	1
Percentages	0·2	2·4	10·6	16·0	18·4	10·6	9·2	9·0	5·6	5·0	4·2	2·0	3·8	1·2	1·2	0·4	—	0·2

Chart 4.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain IV, Hartebeeste, taken on nine consecutive days from Rat 849.

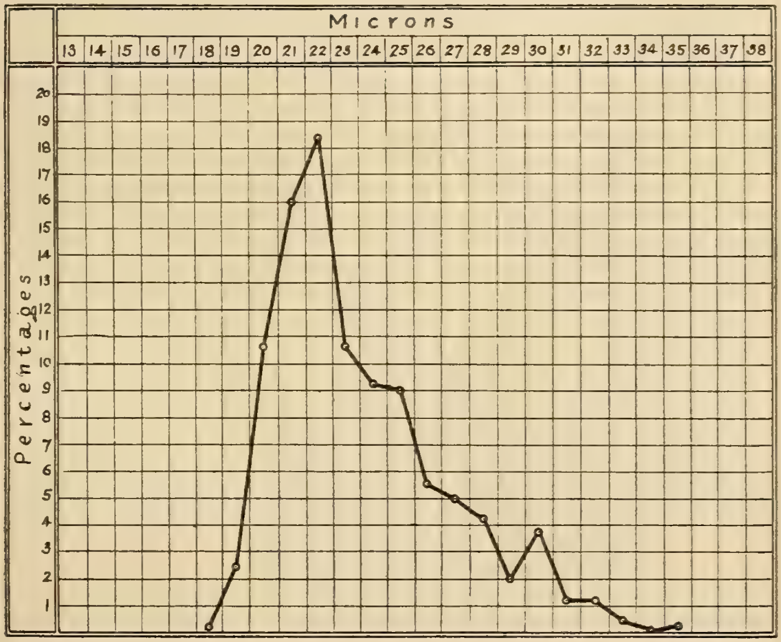


Table XII.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain IV, Hartebeeste.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
July 19	849	Rat	15
„ 20	849	„	18
„ 22	849	„	8
„ 23	849	„	16
„ 24	849	„	26
„ 26	849	„	49
„ 29	849	„	16
„ 30	849	„	38
„ 31	849	„	52
Aug. 1	849	„	45
		Average	28·3

V. *Morphology of Strain V, Hartebeeste.*

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest :—

Table XIII.—Measurements of the Length of the Trypanosome of Strain V, Hartebeeste.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid.	Giemsa	22·6	34·0	15·0

Table XIV.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain V, Hartebeeste.

	In microns.									
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.
Total	1	1	11	30	47	79	51	51	44	37
Percentages	0·2	0·2	2·2	6·0	9·4	15·8	10·2	10·2	8·8	7·4

	In microns.									
	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.
Total	36	35	28	24	11	3	8	1	—	2
Percentages	7·2	7·0	5·6	4·8	2·2	0·6	1·6	0·2	—	0·4

Chart 5.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain V, Hartebeeste, taken on nine consecutive days from Rat 1022.

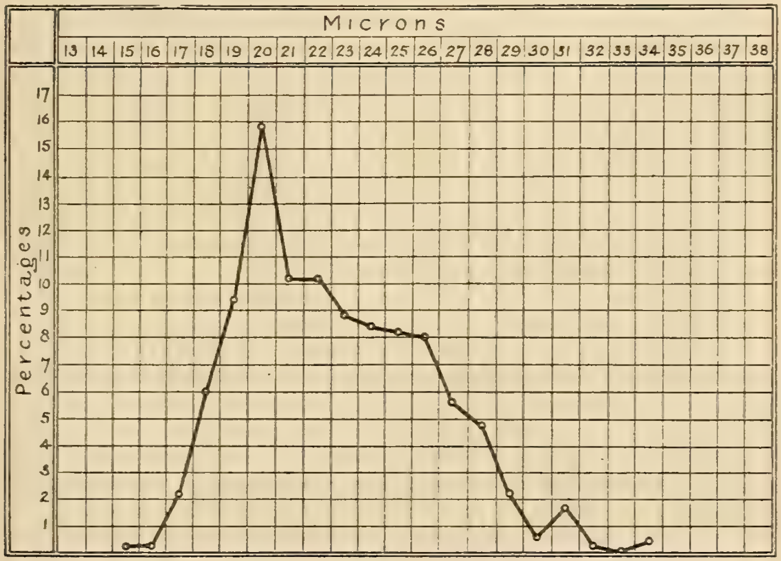


Table XV.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain V, Hartebeeste.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
Aug. 21	1022	Rat	18
„ 22	1022	„	7
„ 23	1022	„	11
„ 24	1022	„	30
„ 26	1022	„	42
„ 27	1022	„	55
„ 28	1022	„	34
„ 29	1022	„	54
Sept. 2	1022	„	50
		Average	33·4

Comparison of the Wild-game Strains with one another.

Table XVI.—Measurements of the Length of the Trypanosome of the Wild-game Strains.

Date.	Experiment No.	Animal.	No. measured.	From what animal.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912	783	Reedbuck	500	Rat	21·7	34·0	16·0
1912	1180	Waterbuck	500	„	23·5	33·0	16·0
1912	863	Oribi	500	„	21·6	33·0	16·0
1912	799	Hartebeeste	500	„	23·5	35·0	18·0
1912	957	„	500	„	22·6	34·0	15·0
			2500	Average	22·6	35·0	15·0

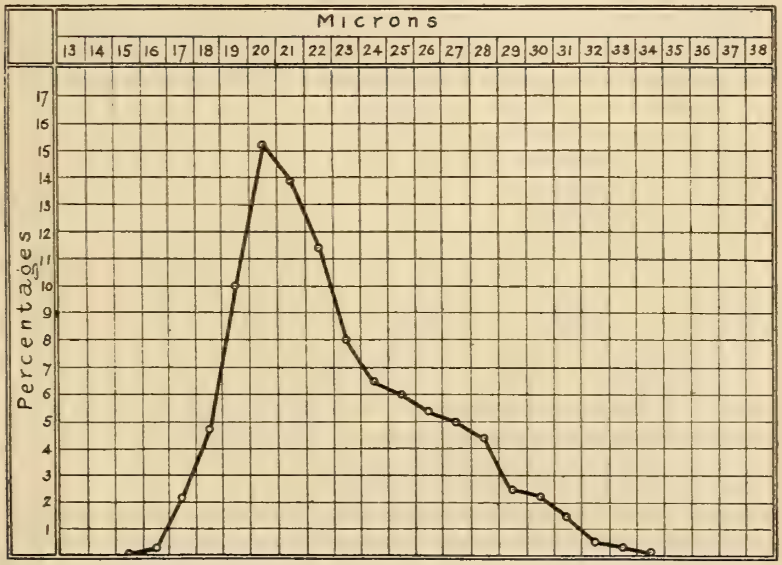
Comparison of the Curves of the Wild-game Strains.

Unlike the curves of the Human strains, these are all remarkably alike, and there can be little doubt that the same species of trypanosome is being dealt with in all five of the Wild-game strains. The Wild-game curves resemble Strains II, IV, and V of the Human strains, described in a former paper, and also those found by Kinghorn and Yorke in the Luangwa Valley.

Table XVII.—Distribution in respect to Length of 2500 Individuals of the Trypanosome of the five Wild-game Strains.

	In microns.																				
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.
Total	1	8	53	118	252	381	348	285	200	162	149	135	125	110	62	55	53	12	7	3	1
Percentages	—	0·3	2·1	4·7	10·0	15·2	13·9	11·4	8·0	6·5	6·0	5·4	5·0	4·4	2·5	2·2	1·5	0·5	0·3	0·1	—

Chart 6.—Composite Curve representing the Distribution, by Percentages, in respect to Length, of 2500 Individuals of the Trypanosome of the Wild-game Strains.



This composite curve resembles the human Strain II, E——.

Table XVIII.—Comparison of the Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosomes of the five Wild-game Strains.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912	783	Reedbuck	8 ·4
1912	1180	Waterbuck	30 ·7
1912	863	Oribi	30 ·3
1912	779	Hartebeeste	28 ·3
1912	957	„	33 ·4
		Average	26 ·2

It is evident from these tables and charts that the various strains of this trypanosome, as they occur in wild game, are remarkably alike. This is what might be expected. Here the trypanosome is at home: it is leading a natural life. It may be supposed to be saved from variation by constantly passing and repassing between the antelope and the tsetse fly.

Comparison of the Human Strain with the Wild-game Strain.

Table XIX.—Average Length of the Trypanosome of the Human and Wild-game Strains.

Strain.	Number of trypanosomes measured.	Animal.	In microns.		
			Average length.	Maximum length.	Minimum length.
Human	3600	Rat	24 ·2	38 ·0	15 ·0
Wild-game	2500	„	22 ·6	35 ·0	15 ·0

The length of the trypanosomes of the Human strain found in white rats only is included in this table, in order to permit of comparison with the Wild-game strain, which is also taken from rats.

The curves (Chart 7) differ from each other in such a marked manner as to be of no use in deciding as to the identity of the Human and Wild-game strains. In spite of this, however, by a comparison of the two strains morphologically and by the susceptibility of the different experimental animals to their pathogenic action, the Commission are driven for the present to the decision that the two strains belong to the same species of trypanosome.

Chart 7.—Curves representing the Distribution, by Percentages, in respect to Length, of 3600 Individuals of the Trypanosome of the Human Strain, and 2500 of the Wild-game Strain.

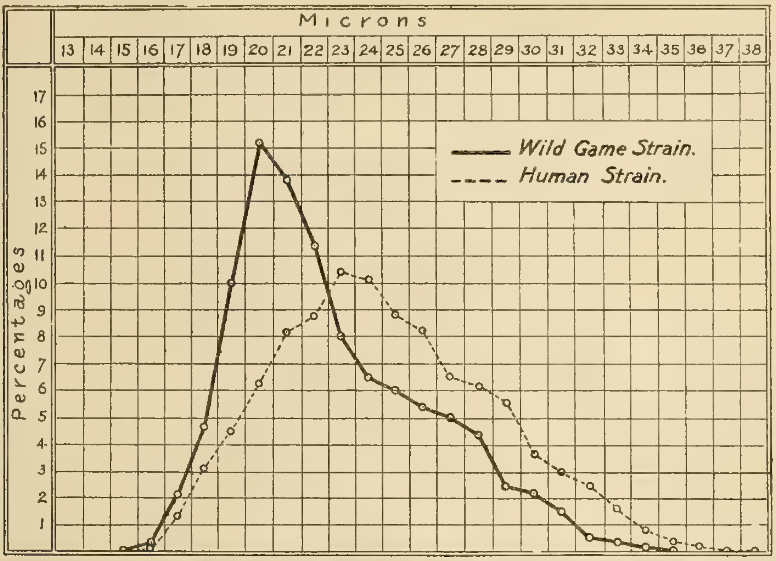


Table XX.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Human and Wild-game Strains.

Date.	Strain.	Average, percentage.	Maximum, percentage.	Minimum, percentage.
1912	Human	21 ·1	52 ·0	2 ·0
1912	Wild-game	26 ·2	33 ·4	8 ·4

Conclusions.

1. The five Wild-game strains resemble each other closely, and all belong to the same species of trypanosome.

2. The Wild-game strains and the Human strains, although they differ to some extent, also belong to the same species.

3. This species is *T. rhodesiense* (Stephens and Fantham).

4. There is some reason for the belief that *T. rhodesiense* and *T. brucei* (Plimmer and Bradford) are one and the same species.

Morphology of Various Strains of the Trypanosome causing Disease in Man in Nyasaland.—The Wild Glossina morsitans *Strain.*

By Surgeon-General Sir David Bruce, C.B., F.R.S., A.M.S.; Majors David Harvey and A. E. Hamerton, D.S.O., R.A.M.C.; and Lady Bruce, R.R.C

(Scientific Commission of the Royal Society, Nyasaland, 1912.)

(Received February 24,—Read April 10, 1913.)

Introduction.

These strains were obtained by bringing tsetse flies (*Glossina morsitans*) to the laboratory from the neighbouring "fly-country" and at once allowing them to feed on healthy animals. The term "wild" is used to distinguish these caught flies from flies bred in the laboratory. The first strain was obtained by feeding the flies on a monkey, the remaining four by feeding on dogs. As soon as the healthy animal was found to be infected other animals were inoculated from it. But, as in the case of the Wild-game strains, only trypanosomes from a single rat were used for purposes of measurement and comparison.

I. *Morphology of Strain I, Wild* Glossina morsitans.

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest.

Table I.—Measurements of the Length of the Trypanosome of Strain I, Wild *Glossina morsitans.*

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	22·7	34·0	16·0

Table II.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain I, Wild *Glossina morsitans.*

	In microns.									
	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.
Total	3	11	25	56	62	75	53	31	28	44
Percentages	0·6	2·2	5·0	11·2	12·4	15·0	10·6	6·2	5·6	8·8

	In microns.								
	26.	27.	28.	29.	30.	31.	32.	33.	34.
Total	16	23	26	22	12	6	4	1	2
Percentages	3·2	4·6	5·2	4·4	2·4	1·2	0·8	0·2	0·4

CHART 1.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain I, Wild *Glossina morsitans*, taken on nine consecutive days from Rat 655.

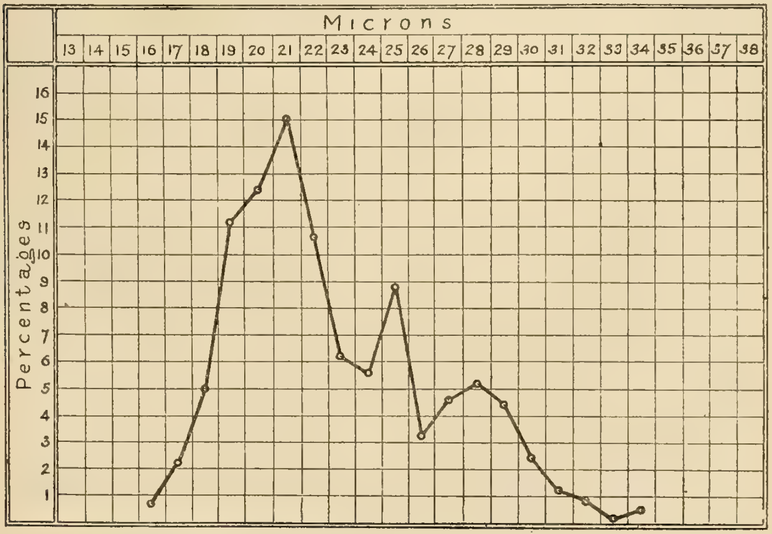


Table III.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain I, Wild *Glossina morsitans.*

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912			
June 18	655	Rat	6
„ 19	655	„	10
„ 20	655	„	11
„ 21	655	„	17
„ 22	655	„	0
„ 24	655	„	16
„ 25	655	„	28
„ 26	655	„	26
„ 27	656	„	23
„ 28	655	„	0
„ 29	655	„	7
„ 30	655	„	4
		Average	12·3

II. *Morphology of Strain II, Wild* Glossina morsitans.

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest.

Table IV.—Measurements of the Length of the Trypanosome of Strain II, Wild *Glossina morsitans.*

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	24·5	34·0	17·0

Table V.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain II, Wild *Glossina morsitans*.

	In microns.								
	17.	18.	19.	20.	21.	22.	23.	24.	25.
Total ……………	1	4	20	43	67	44	48	34	42
Percentages ……	0·2	0·8	4·0	8·6	13·4	8·8	9·6	6·8	8·4

	In microns.								
	26.	27.	28.	29.	30.	31.	32.	33.	34.
Total ……………	35	33	43	28	23	19	13	2	1
Percentages ……	7·0	6·6	8·6	5·6	4·6	3·8	2·6	0·4	0·2

Chart 2.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain II, Wild *Glossina morsitans*, taken on nine consecutive days from Rat 656.

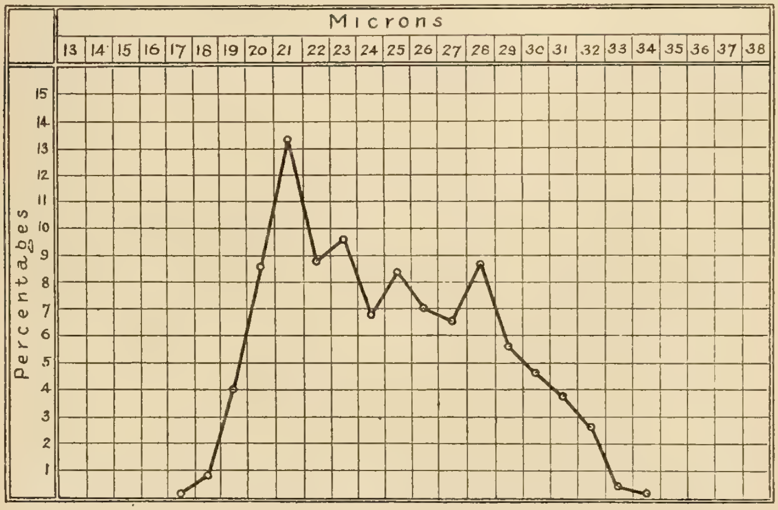


Table VI.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain II, Wild *Glossina morsitans.*

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912			
June 17	656	Rat	8
„ 18	656	„	12
„ 19	656	„	13
„ 20	656	„	22
„ 21	656	„	43
„ 22	656	„	44
„ 24	656	„	27
„ 25	656	„	28
„ 26	656	„	45
„ 27	656	„	47
		Average	28·9

III. *Morphology of Strain III, Wild* Glossina morsitans.

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest.

Table VII.—Measurements of the Length of the Trypanosome of Strain III, Wild *Glossina morsitans.*

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	20·5	31·0	16·0

Table VIII.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain III, Wild *Glossina morsitans.*

	In microns.															
	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.
Total	19	72	84	85	44	24	40	33	27	18	21	12	11	3	4	3
Percentages	3·8	14·4	16·8	17·0	8·8	4·8	8·0	6·6	5·4	3·6	4·2	2·4	2·2	0·6	0·8	0·6

Chart 3.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain III, Wild *Glossina morsitans*, taken on nine consecutive days from Rat 657.

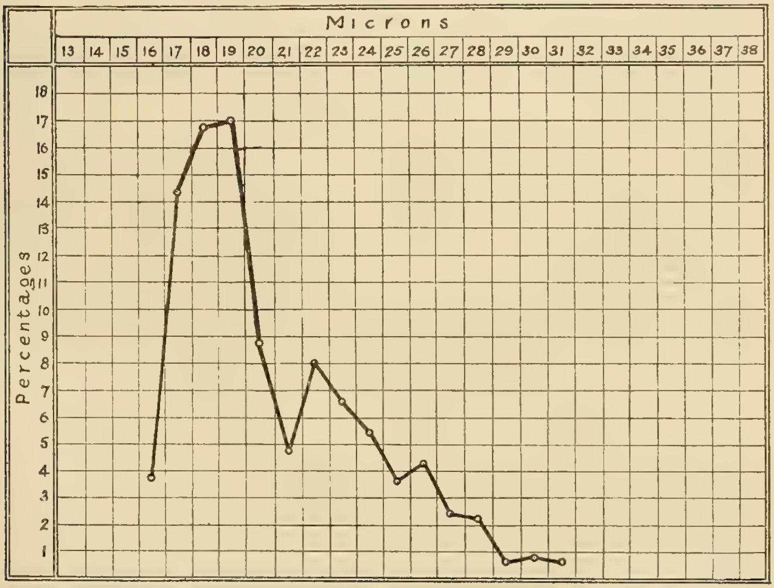


Table IX.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain III, Wild *Glossina morsitans.*

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
June 17	657	Rat	4
„ 18	657	„	9
„ 19	657	„	13
„ 20	657	„	4
„ 21	657	„	2
„ 22	657	„	15
„ 24	657	„	32
„ 25	657	„	18
„ 26	657	„	4
„ 27	657	„	27
		Average	12 ·8

IV. *Morphology of Strain IV, Wild* Glossina morsitans.

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest.

Table X.—Measurements of the Length of the Trypanosome of Strain IV, Wild *Glossina morsitans.*

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	22 ·7	34 ·0	15 ·0

Table XI.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain IV, Wild *Glossina morsitans.*

	In microns.									
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.
Total	1	5	37	60	71	54	34	25	17	13
Percentages	0·2	1·0	7·4	12·0	14·2	10·8	6·8	5·0	3·4	2·6
	In microns.									
	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.
Total	15	19	34	30	31	30	8	14	1	1
Percentages	3·0	3·8	6·8	6·0	6·2	6·0	1·6	2·8	0·2	0·2

Chart 4.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain IV, Wild *Glossina morsitans*, taken on nine consecutive days from Rat 658.

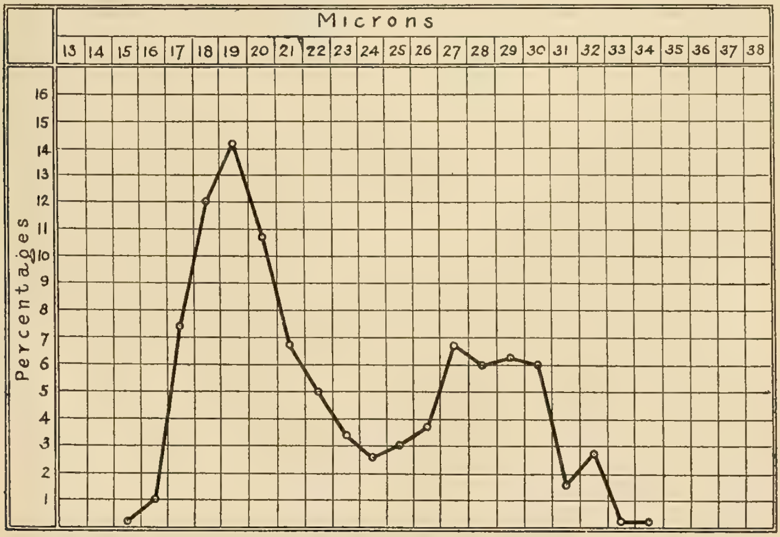


Table XII.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain IV, Wild *Glossina morsitans.*

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
June 17	658	Rat	0
„ 18	658	„	0
„ 19	658	„	6
„ 21	658	„	16
„ 22	658	„	9
„ 24	658	„	7
„ 25	658	„	7
„ 26	658	„	8
„ 27	658	„	0
„ 28	658	„	10
„ 29	658	„	0
July 1	658	„	0
„ 2	658	„	0
„ 3	658	„	0
		Average............	4·5

V. *Morphology of Strain V, Wild* Glossina morsitans.

The following table gives the average length of this trypanosome as found in the rat, 500 trypanosomes in all, and also the length of the longest and shortest:—

Table XIII.—Measurements of the Length of the Trypanosome of Strain V, Wild *Glossina morsitans.*

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	22·8	35·0	15·0

Table XIV.—Distribution, in respect to Length, of 500 Individuals of the Trypanosome of Strain V, Wild *Glossina morsitans.*

	In microns.									
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.
Total	6	4	27	57	94	49	37	22	14	13
Percentages	1·2	0·8	5·4	11·4	18·8	9·8	7·4	4·4	2·8	2·6

	In microns.										
	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.
Total	11	19	25	23	29	27	18	13	7	3	2
Percentages	2·2	3·8	5·0	4·6	5·8	5·4	3·6	2·6	1·4	0·6	0·4

Chart 5.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain V, Wild *Glossina morsitans*, taken on nine consecutive days from Rat 660.

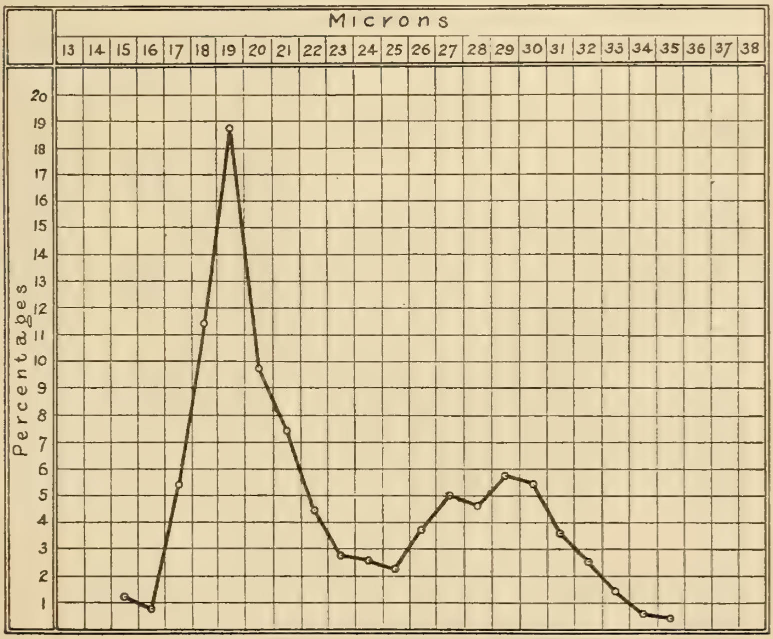


Table XV.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain V, Wild *Glossina morsitans.*

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
June 18	660	Rat	0
,, 19	660	,,	0
,, 20	660	,,	0
,, 21	660	,,	8
,, 22	660	,,	19
,, 24	660	,,	0
,, 25	660	,,	6
,, 26	660	,,	4
,, 27	660	,,	6
,, 28	660	,,	6
,, 29	660	,,	2
July 2	660	,,	2
,, 3	660	,,	1
		Average	4·2

Comparison of the Wild Glossina morsitans *Strains with one another.*

Table XVI.—Measurements of the Length of the Trypanosome of the Wild *Glossina morsitans* Strains.

Date.	Expt. No.	Animal.	Number measured.	From what animals.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912	523	Monkey	500	Rat	22·7	34·0	16·0
1912	542	Dog	500	,,	24·5	34·0	17·0
1912	551	,,	500	,,	20·5	31·0	16·0
1912	595	,,	500	,,	22·7	34·0	15·0
1912	602	,,	500	,,	22·8	35·0	15·0
			2500	Average...	22·6	35·0	15·0

Table XVII.—Distribution in respect to Length of 2500 Individuals of the Trypanosome of the five Wild *Glossina morsitans* Strains.

	In microns.									
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.
Total............................	7	31	148	230	326	252	237	184	143	115
Percentages	0·3	1·2	5·9	9·2	13·1	10·1	9·5	7·3	5·7	4·6

	In microns.										
	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.
Total............................	130	110	127	133	113	96	54	44	11	7	2
Percentages	5·2	4·4	5·1	5·3	4·5	3·8	2·2	1·8	0·4	0·3	0·1

Chart 6.—Curve representing the Distribution, by Percentages, in respect to Length, of 2500 Individuals of the Trypanosome of the Wild *Glossina morsitans* Strains.

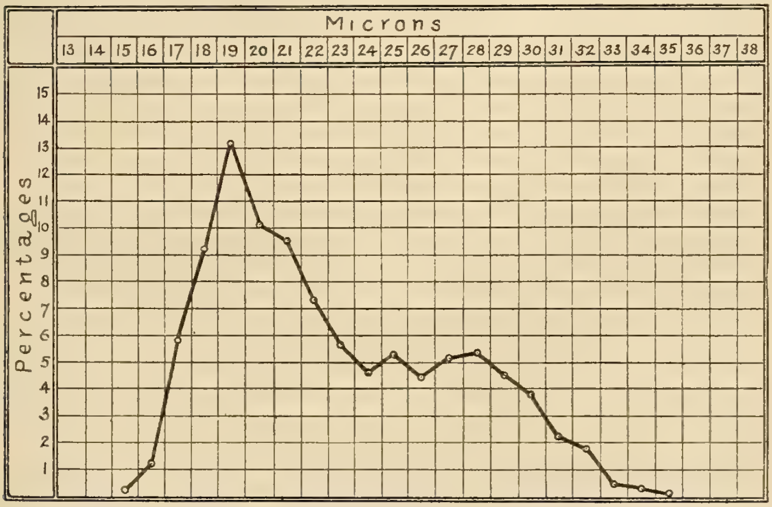


Table XVIII.—Comparison of the Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the five Wild *Glossina morsitans* Strains.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912	523	Monkey	12·3
1912	542	Dog	28·9
1912	551	,,	12·8
1912	595	,,	4·5
1912	602	,,	4·2
		Average	12·5

Comparison of the Human Strain with the Wild-game and Wild Glossina morsitans *Strains.*

Table XIX.—Average Length of the Trypanosome of the Human, Wild-game, and Wild *Glossina morsitans* Strains.

Strain.	Number of trypanosomes measured.	Animal.	In microns.		
			Average length.	Maximum length.	Minimum length.
Human	3600	Rat	24·2	38·0	15·0
Wild-game	2500	,,	22·6	35·0	15·0
Wild *G. morsitans*	2500	,,	22·6	35·0	15·0

It is remarkable that the Wild-game strains and the Wild *Glossina morsitans* strains should come out exactly alike. Why the Human strains differ to some extent from the other two cannot at present be explained.

The curves of the Wild-game strains and the Wild *Glossina morsitans* strains are so similar that there can be little doubt the same species of trypanosome is being dealt with. The Human strain differs so much, that the suspicion must present itself that in some way more than one species is being dealt with. The three Human strains which differ most from the Wild-game and Wild *Glossina morsitans* type are Stephens and Fantham's case of Armstrong, in Liverpool; Strain I, Mkanyanga; and Strain III, Chituluka. On examining these three strains, however, from every possible point of view, nothing, except the difference in the type of curve, can be found to justify this suspicion.

Chart 7.—Curves representing the Distribution, by Percentages, of 3600 Individuals of the Trypanosome of the Human Strains, 2500 of the Wild-game Strains, and 2500 of the Wild *Glossina morsitans* Strains.

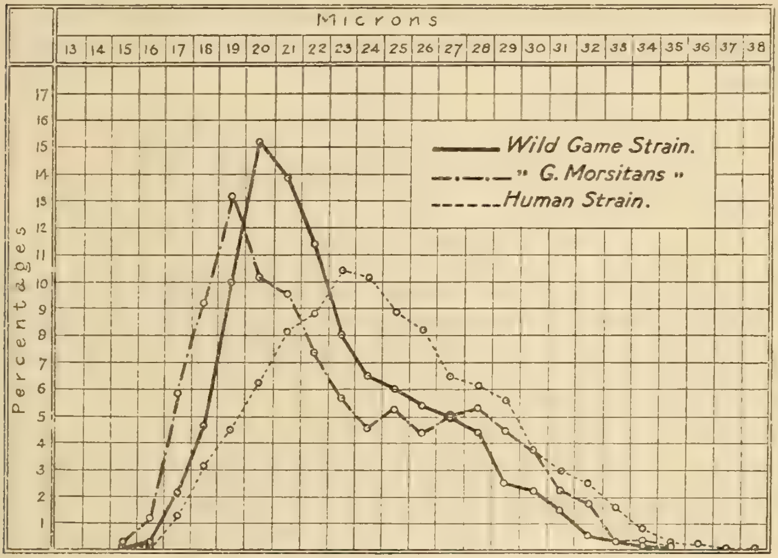


Table XX.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Human, Wild-game, and Wild *Glossina morsitans* Strains.

Date.	Strain.	Percentages.		
		Average.	Maximum.	Minimum.
1912	Human	21 ·1	52 ·0	2 ·0
1912	Wild-game	26 ·2	33 ·4	8 ·4
1912	Wild *G. morsitans*	12 ·5	28 ·9	4 ·2

Conclusions.

1. The five Wild *Glossina morsitans* strains resemble each other closely, and all belong to the same species of trypanosome.

2. The Wild *Glossina morsitans* strain, the Human strain, and the Wild-game strain, belong to the same species.

3. This species is *T. rhodesiense* (Stephens and Fantham).

4. It is probable that *T. rhodesiense* and *T. brucei* (Plimmer and Bradford) are identical.

Infectivity of Glossina morsitans *in Nyasaland.*

By Surgeon-General Sir David Bruce, C.B., F.R.S., A.M.S.; Majors David Harvey and A. E. Hamerton, D.S.O., R.A.M.C.; and Lady Bruce, R.R.C.

(Scientific Commission of the Royal Society, Nyasaland, 1912.)

(Received February 24,—Read April 10, 1913.)

Introduction.

The object of this paper is to put on record the proportion of tsetse flies found in this district of Nyasaland to be infected with disease-producing trypanosomes, and, further, to identify the species of trypanosomes with which the flies are infected.

It must be understood that this paper only deals with the district lying near the camp at Kasu and not to every part of Nyasaland. The geographical position and other features of this district have already been described in a previous paper.* It is known as the "Sleeping Sickness Area" or "Proclaimed Area," and in it almost all the cases of the Human Trypanosome Disease of Nyasaland have occurred. This disease is caused by *Trypanosoma rhodesiense,* and the natives give it the name of "Kaodzera," but there is a good deal of evidence accumulating that *T. rhodesiense* is in reality *T. brucei,* in which case the disease would be known as "Nagana."

Definitions.

In this paper an *infective* fly is one which contains trypanosomes which have reached the final stage of their development and are capable of giving rise to disease. An *infected* fly is one in which the development of the trypanosomes may not have reached this final stage, and where, therefore, it may not as yet be disease-producing. An infective fly must be infected, whereas an infected fly need not be infective.

"Fly" means the tsetse fly, and in this paper the species dealt with is *Glossina morsitans.*

Methods Employed.

The method employed in studying the infectivity of the flies was simple. Native boys were employed in catching the flies, which were brought up to Kasu in small cages by a native cyclist. Each cage of flies was fed on three healthy animals—the first day on a monkey, the second on a dog, and the third on a goat. To ensure, as far as possible, that each animal was fed on by every fly, the flies were fed nine times—three times on each animal.

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* *Supra,* p. 269.

The number of flies brought up each day would probably average about 60, and as each animal was fed on by three cagefuls, then each monkey, dog, and goat ran the gauntlet of some 180 flies. It is therefore impossible to arrive at any very precise knowledge of the proportion of infective flies in each cage.

Infectivity of the Flies.

As will be seen from Table I, every experiment, with the exception of one, was positive, and on two occasions a goat was infected by all the four species of pathogenic trypanosomes occurring in this neighbourhood.

The Commission showed in a previous paper* that one in three of the wild game found in this district is infected with trypanosomes, and recommended that the animals should be destroyed. If this were done and a year allowed to elapse, the proportion of infective flies then found would be an index of the usefulness or futility of such operations.

The Commission is of opinion that the wild game is the principal factor in the spread of trypanosome disease, and that, for practical purposes, the smaller mammals, birds, and reptiles need not be taken into account.

The following table gives, in the first column, the date the first cageful of flies was fed on the monkey, the second column the number of flies fed; the signs *plus* and *minus* show the result of feeding the flies on the monkey, dog, and goat.

The four species of trypanosomes carried by the "fly" in this district are *T. brucei* vel *rhodesiense*, *T. pecorum*, *T. simiæ*, and *T. capræ*. The first and second of these attack all three animals, the third the monkey and goat, being harmless to the dog, whereas the fourth only produces disease in the goat.

Where no *plus* or *minus* sign occurs it means that an animal was not available. For example, the experiment beginning on January 20 shows that the monkey was infected by *T. simiæ*, the goat by *T. brucei*, and that no dog was available. The experiment on February 21 shows that neither the dog nor goat became infected by the bites of 170 flies, and that no monkey was available.

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* *Supra*, p. 277.

Table I.—The Result of Feeding 10,081 Tsetse Flies (*G. morsitans*), caught in the "Proclaimed Area," Nyasaland, on Monkeys, Dogs, and Goats.

1912.	Number of flies fed.	Monkey.				Dog.				Goat.			
		T. brucei vel *T. rhodesiense.*	*T. pecorum.*	*T. simiæ.*	*T. capræ.*	*T. brucei* vel *T. rhodesiense.*	*T. pecorum.*	*T. simiæ.*	*T. capræ.*	*T. brucei* vel *T. rhodesiense.*	*T. pecorum.*	*T. simiæ.*	*T. capræ.*
Jan. 20	296	—	—	+	—					+	—	—	—
,, 24	370	—	—	+	—					—	+	—	+
,, 29	280	—	—	+	—					—	+	+	—
Feb. 2	295	—	—	+	—					—	+	+	+
,, 8	220	—	—	+	—					—	+	+	+
,, 13	200	—	+	+	—					—	—	—	+
,, 16	195					+	—	—	—	+	—	—	—
,, 21	170					—	—	—	—	—	—	—	—
,, 26	170					—	—	—	—	—	—	+	—
Mar. 2	140					—	—	—	—	—	—	—	+
,, 9	165					—	+	—	—	—	—	—	+
,, 14	100					—	—	—	—	—	—	—	+
,, 17	160					—	+	—	—	—	+	—	—
,, 22	205					—	+	—	—	+	+	+	+
Apr. 3	135					—	+	—	—	—	+	—	—
,, 10	275	+	—	+	—	+	+	—	—	—	—	—	+
,, 15	330	—	—	+	—	—	+	—	—	—	+	+	+
,, 18	200	—	—	+	—	—	+	—	—	—	+	—	+
,, 18	180	—	—	+	—	—	+	—	—	—	—	+	—
,, 23	230	—	—	+	—	—	+	—	—	—	—	—	+
,, 23	140	—	+	—	—	—	+	—	—	—	+	—	+
,, 26	100	—	—	+	—	—	+	—	—	+	+	+	—
,, 27	260	—	—	+	—	—	+	—	—	—	+	—	+
May 3	155	+	+	+	—	—	+	—	—	—	—	—	+
,, 3	96	—	—	—	—	—	+	—	—	—	+	—	—
,, 8	330	+	—	+	—	+	—	—	—	—	+	—	+
,, 9	120	—	—	+	—	—	+	—	—	—	+	—	+
,, 13	50	—	—	—	—	+	—	—	—	—	—	+	+
,, 14	250	—	—	+	—	—	+	—	—	+	+	+	+
,, 17	190					+	+	—	—	—	—	+	+
,, 24	113					—	+	—	—	—	+	+	+
,, 29	120	—	—	—	—	—	+	—	—	—	+	—	—
,, 29	230	—	—	—	—	—	+	—	—	—	+	—	—
,, 29	320					+	+	—	—	—	+	—	+
,, 29	240	—	+	—	—	—	+	—	—	—	+	+	+
,, 29	100					—	+	—	—	—	+	+	+
,, 31	175	+	—	+	—	+	+	—	—	—	+	+	—
June 2	300					—	+	—	—	—	—	+	+
,, 6	210	—	+	—	—	—	+	—	—	—	+	—	+
,, 7	230	+	—	—	—	+	+	—	—	+	+	—	—
,, 11	160	—	—	+	—	—	+	—	—	—	+	—	+
,, 18	135	—	—	—	—	—	+	—	—	—	+	—	—
,, 25	90	+	—	—	—	+	—	—	—	—	+	—	+
July 3	95					—	+	—	—	—	+	—	—
Sept. 25	70	—	+	—	—								
,, 27	25	+	—	—	—								
Oct. 29	87	+	—	—	—	+	—	—	—	+	+	—	+
Nov. 5	145					—	—	—	—	—	—	—	+
,, 11	150	—	—	+	—	—	+	—	—	—	+	—	+
,, 18	157	—	—	+	—	—	+	—	—	—	—	+	+
,, 21	95	—	—	—	—	+	—	—	—	+	—	—	—
,, 25	180	—	—	+	—	+	—	—	—	+	+	—	—
Dec. 3	180	—	+	+	—	—	+	—	—	—	+	—	+
,, 6	198	+	+	+	—	+	—	—	—	+	—	—	+
,, 11	156	—	—	+	—	+	—	—	—	+	+	—	—
,, 16	113	—	+	+	—	—	+	—	—	—	+	—	+
Total......	10,081	9	9	26		14	34			11	35	17	35

Table II.—The Number of Times a Monkey, Dog, and Goat became infected with *Trypanosoma brucei* vel *rhodesiense*, *T. pecorum*, *T. simiæ*, and *T. capræ* in a Series of 56 Experiments, averaging 180 Tsetse Flies each.

T. brucei vel *rhodesiense.*			*T. pecorum.*			*T. simiæ.*			*T. capræ.*		
Monkey.	Dog.	Goat.	Monkey.	Dog.	Goat.	Monkey.	Dog.	Goat.	Monkey.	Dog.	Goat.
9	14	11	9	34	35	26	0	17	0	0	35

This shows that the monkey is less susceptible to *T. brucei* and *T. pecorum* than the dog, whereas it is remarkably so to *T. simiæ*. The dog is not susceptible to *T. simiæ*, and neither the monkey nor dog to *T. capræ*.

Table III.—The Proportion per 1000 Tsetse Flies, caught in the "Sleeping-Sickness" Area of Nyasaland, found to be Infective with Pathogenic Trypanosomes.

T. brucei vel *rhodesiense.*	*T. pecorum.*	*T. simiæ.*	*T. capræ.*
Per 1000. 2·0	Per 1000. 4·6	Per 1000. 3·4	Per 1000. 3·5

This is only allowing one infective fly to each series of flies fed on the experimental animals, and is therefore the irreducible minimum. The average number of flies fed on each animal was 180, and it might well be that there were present in the same batch several flies infective with the same species of trypanosome. Ten thousand flies gave rise to 135 infections, and taking it for granted that no fly was infective with more than one species of trypanosome, then 13·5 per 1000 flies are infective with one or other of the disease-producing trypanosomes of this district.

Table IV.—Number of Times the Species of Trypanosomes under consideration were found in 56 Experiments.

T. brucei vel *rhodesiense.*	*T. pecorum.*	*T. simiæ.*	*T. capræ.*
20	46	34	35
35·7 per cent.	82·1 per cent.	60·7 per cent.	62·5 per cent.

This means that in experiments carried out in the manner described *T. brucei* may be expected to turn up once in every three series, *T. pecorum* eight times in ten, and *T. simiæ* and *T. capræ* six times in ten.

Months and Seasons.

On examining Table I it will be seen that these infective flies occur all the year round, and are just as numerous during one season as another. It will also be seen that no experiments on the infectivity of the flies were carried out during July and August. This was due to the fact that all the energy of the Commission was devoted during these two months to the study of the wild game.

Conclusions.

1. The tsetse flies (*Glossina morsitans*) caught in the "fly-country" near Kasu are infected with four species of disease-producing trypanosomes—*T. brucei* vel *rhodesiense*, *T. pecorum*, *T. simiæ*, and *T. capræ*.
2. The proportion of infective flies is 13·5 per 1000.
3. The proportion of flies infective with *T. brucei* vel *rhodesiense*, the cause of the Human Trypanosome Disease of Nyasaland, is 2 per 1000.
4. The flies are found infective all the year round.
5. To prevent the infection of tsetse flies it is proposed that the experiment should be tried of destroying all the wild game in the "Proclaimed Area" of Nyasaland.

The Excystation of Colpoda cucullus *from its Resting Cysts, and the Nature and Properties of the Cyst Membranes.*

By T. Goodey, M.Sc. (Birm.), late Mackinnon Student of the Royal Society.

(Communicated by A. D. Hall, F.R.S. Received February 28,—
Read May 8, 1913.)

(From the Rothamsted Experimental Station, Harpenden.)

Introduction.—Having had occasion to make use of the resting cysts of *Colpoda cucullus* in the course of my work on the protozoa of the soil* I was interested by the way in which the organisms escape from the confines of the cyst membranes. The processes involved were by no means obvious on somewhat casual observation, and it became necessary to study them in considerable detail before they could be fully elucidated. Moreover it was thought that by working out as fully as possible the conditions involved in excystation some light might be thrown on the activity or inactivity of the protozoa in the soil.

The water-content, available food supply, and temperature of any soil are obvious external factors in determining the possibility of protozoal activity, but that these were all the determining factors was by no means clear. There was the possibility that certain peculiar external influences were required for excystation, and if these could be determined it was possible that one would be able to say whether they were present or absent in a soil normally containing protozoa.

In studying the literature bearing on the subject it was soon evident that although a considerable amount of work had been done by earlier workers, particularly by Rhumbler† on the excystation of *Colpoda* and the different kinds of cysts formed by it, and by Fabre‡ on the properties of cyst membranes, little or no work was recorded dealing with the processes involved in the emergence of active forms from resting cysts.

I therefore set out to investigate the matter as fully as possible.

Methods.—The experiments have been carried out in hanging-drop preparations. A drop of hay-infusion or other liquid medium* is taken on a loop of platinum wire and spread out on a clean cover-slip; into the liquid is placed a number of cysts of *Colpoda,* and the cover-slip is then inverted over the hollow of a cavity slide and the edges waxed down by painting round with the hot wick of a candle. It is then allowed to incubate. In order to experiment further with the excysted organisms, the cover-slip is carefully lifted by means of a needle, and after the necessary treatment it is replaced and the edge waxed down again. Where not otherwise stated incubation has been carried out at 30° C., this being the temperature at which excystation is most rapid.

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* Goodey, T., 'A Contribution to our Knowledge of the Protozoa of the Soil," 'Roy. Soc. Proc.,' 1911, B, vol. 84, p. 165.

† Rhumbler, L., 'Zeitschr. für wissen. Zool.,' 1888, vol. 46, p. 571.

‡ Fabre-Domergue, P., "Sur les propriétés dialytiques de la membrane du Kyste des Infusoires," 'Compt. Rend.,' 1885, vol. 101; "Recherches anat. et physiol. sur les Infusoires ciliés," 'Ann. des Sci. Nat.' (7 s.), 'Zoologie,' 1888, vol. 5.

Material.—The investigations have been principally carried out with the resting cysts of *Colpoda cucullus.* A culture of this species, of a particular strain measuring about 45 μ in length and having a rather pointed anterior end, was obtained free from any other ciliated protozoa. By making sub-cultures of this from time to time in sterile 1-per-cent. hay-infusion, and allowing the *Colpoda* to go on multiplying and encyst, quantities of resting cysts were obtained. For convenience the cysts were collected on small filter papers and kept in an air-tight glass dish. A quantity of cysts of a larger strain of *Colpoda cucullus* was also obtained, but these did not prove to be so convenient for manipulation as the smaller strain.

It was found that if the cysts were kept for some weeks on the filter paper in a quite dry condition, their power of excysting rapidly was considerably diminished. Excystation was most rapid in the case of cultures made from cysts recently filtered and almost air dried.

Influence of Temperature on Rate of Excystation.—Hanging-drop cultures were made with cysts in hay-infusion and put into incubators at different temperatures, 40°, 30°, 25°, and 20° C. The cysts were all from the same collection and the hay-infusion from the same stock solution, so that conditions were all similar, except for temperature.

The results were as follows:—

40° C., none excysted after several hours.
30° C., many active after 1 hour incubation.
25° C., a few active after 1 hour 17 minutes.
20° C., a few active after 2 hours 12 minutes.

It is evident that differences in temperature have a considerable influence on the rate of excystation. It was not possible to determine exactly the optimum temperature for excystation, owing to there being no incubators at other temperatures, but it lies close to 30° C.

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* Distilled water, tap-water, and aqueous soil-extract were used at different times. Excystation takes place quite freely in distilled water.

Meunier* obtained active *Colpoda* two hours after moistening resting cysts, and Fabre† also obtained active forms in the same time.

Influence of Alkaline, Acid, and Neutral Media on Excystation.—The ordinary 1-per-cent. hay-infusion used for cultures of *Colpoda* and other protozoa is made slightly alkaline in reaction to litmus by the addition of caustic soda solution and contains 0·01 per cent. NaOH. *Colpoda cucullus* excysts very freely in this liquid and also in neutral 1-per-cent. hay-infusion. When, however, the hay-infusion was made slightly acid in reaction to litmus, excystation appeared to be completely inhibited, and from these preliminary trials the inference was tentatively drawn, that excystation was inhibited in an acid medium.

It was necessary, however, to determine more accurately the critical percentage strength of acid and alkali at which excystation is inhibited.

NaOH.—1-per-cent. hay-infusion was put up in 10 c.c. lots, each with a different percentage strength of NaOH, starting at 0·01 per cent. and going up in hundredths of 1 c.c. to 0·2 per cent.

Excystation was rapid and free in all the cultures below 0·15 per cent. NaOH. At this strength, however, only a few were active after two hours' incubation and these were apparently rather uncomfortable. At 0·16 per cent. one or two were trying to excyst, 0·17 per cent. showed one or two revolving within their endocysts but unable to get out quickly, at 0·18 per cent. there was no motion, though the contractile vacuoles in one or two were dilated, 0·19 per cent. gave the same appearance as 0·18 per cent., and at 0·2 per cent. there was no indication whatever of excystation.

After 21 hours' incubation active forms were found in all, up to and including 0·18 per cent. and 0·19 per cent., but none were active in 0·2 per cent. This then is the critical percentage strength of NaOH which inhibits excystation, whilst 0·18 per cent. may be taken as the critical strength for short-period incubation.

HCl.—For hydrochloric acid a number of 10 c.c. lots of 1-per-cent. hay-infusion were put up, each with a different percentage strength of HCl, starting at 0·001 per cent. and going up to 0·01 per cent. in thousandths of 1 c.c. and then in hundredths of 1 c.c. up to 0·1 per cent.

After one hour's incubation at 30° C. there were many active in all the cultures up to 0·01 per cent. At 0·08 per cent. there were a few active, at 0·09 per cent. only one or two, and at 0·1 per cent. none were active; 0·1-per-cent. HCl is then the critical strength which inhibits excystation. *Colpoda* excystation can therefore take place within fairly wide limits in an alkaline medium containing 0·18 per cent. or 0·19 per cent. NaOH and in the presence of 0·09 per cent. HCl.

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* Meunier, V., "Sur la résistance vit. des Kolpodes 'encystés,'" 'Compt. Rend.,' 1865, vol. 61.

† Fabre-Domergue, P., *loc. cit.*

Tests for the Nature of the Ecto- and Endo-cysts.

Fabre found that the ectocysts of resting cysts could withstand the action of concentrated sulphuric acid for a long time, and also caustic potash solution. He failed to obtain a cellulose reaction with iodine and sulphuric acid, whereas Stein obtained a wine-red coloration in this manner with *Vorticella microstoma*, and believed that the cyst-membranes of this organism were composed of a substance combined with cellulose, which could be dissolved out by the action of caustic potash.

In the course of the present investigation the following tests have been applied in order to determine the nature and characteristics of the ectocyst and endocyst membranes of *Colpoda cucullus.*

Solubility in Water.—Both ectocyst and endocyst are insoluble in cold water and in water or 1-per-cent. hay-infusion at 95–100° C.

Acids.—Sulphuric acid, strong, does not affect either ectocyst or endocyst in the cold. Acetic acid, 90 per cent., has no action on ectocyst or endocyst in the cold. Hydrochloric acid, strong and cold, causes the ectocyst to swell up slightly, but does not dissolve the endocyst. On gradually heating up cultures containing ectocysts and endocysts with 2-per-cent. hydrochloric acid, and keeping the temperature at about 97° C. for half an hour, the endocysts disappeared, whilst the ectocysts remained somewhat swollen.

Alkalies.—Caustic soda: 1 per cent., 2 per cent., and 4 per cent. do not dissolve ectocysts or endocysts in the cold, though they penetrate freely into the endocysts and attack the *Colpoda* within, causing them to swell up and become transparent. At 30° C., 1 per cent. and 2 per cent. still do not attack the endocyst membrane, though 4 per cent. causes its solution at this temperature. Twenty-per-cent. caustic soda, acting in the cold, causes the ectocyst to swell up considerably and become transparent, though there still remain indications of the layers making up this membrane. Caustic potash: 1 per cent. and 2 per cent. do not dissolve ectocysts or endocysts in the cold.

Fat Solvents: Alcohol, 95 per cent., ether, toluene, and chloroform do not dissolve the ectocyst or endocyst when added to a culture which has been exposed to the action of osmic vapour for a few seconds in order to kill the active and excysting organisms.

Formalin: 40-per-cent. formalin does not dissolve either ectocyst or endocyst in the cold. Prowazek* mentions that Kutscher found paramylum was soluble in formalin.

Protein Tests.—Xanthoproteic test: cultures containing ectocysts and endocysts were carefully heated with concentrated nitric acid and then ammonia was added, but no coloration resulted. Millon's reagent was used on two or three occasions, but there was no red coloration of the cyst-membranes, though by this method protein was detected in the protoplasm of *Colpoda* by the brick-red coloration.

Starch and Cellulose Tests.—Iodine in potassium iodide solution does not stain ectocysts or endocysts, though it passes through the latter very readily and stains the *Colpoda* light brown. Preparations treated with iodine are not affected when strong sulphuric acid is added. Ammoniacal cupric hydrate does not dissolve either ectocyst or endocyst. Corallin soda solution does not stain the endocyst pink. These negative reactions show that the cyst-membranes are not composed of starch or cellulose.

The following carbohydrates occur in certain protozoa:—

Glycogen† has been observed in a number of ciliated protozoa, *Opalina*, *Paramœcium*, and *Vorticella*. It stains light or reddish brown with iodine.

Paraglycogen‡ occurs as refractive spherules in certain of the *Sporozoa*. With iodine it stains brown, which changes to wine-red or violet on the addition of 70 per cent. sulphuric acid. It is soluble in hot water.

Paramylum§ is a carbohydrate nearly related to cellulose. As prepared from *Euglena viridis* by Bütschli it did not stain with iodine in potassium iodide solution nor was it affected by the addition of 70-per-cent. sulphuric acid. It is insoluble in cold and hot water and is hydrolysed by continued boiling with strong sulphuric acid.

It is evident that the cyst membranes of *Colpoda* are not composed of glycogen or paraglycogen. They resemble paramylum in their reactions to iodine and in the fact that the endocyst disappears on being heated up with acid. It will be shown later on, however, that they are not composed of paramylum.

Reactions to Stains.—The following substances were tried in order to determine what staining reactions are given by the cyst-membranes:—

Picric acid, a strong aqueous solution: membranes not stained.

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* Prowazek, S. von, 'Einführung in die Physiologie der Einzelligen (Protozoen),' Berlin, 1910, p. 13.

† Prowazek, S. von, *loc. cit.*; Bütschli, C., *loc. cit.*, pp. 1469–72.

‡ Bütschli, O., *loc. cit.*, pp. 1469–72; Minchin, E. A., "The Sporozoa," 'A Treatise on Zoology,' Pt. 1, 2nd Fas., 1903, p. 182.

§ Bütschli, O., "Kenntniss des Paramylons," 'Arch. für Protist.,' 1906, vol. 7, p. 199.

Methyl green, saturated aqueous solution + 1 per cent. acetic acid: membranes not stained, contents of endocyst immediately stained.

Iodine green: same result as with methyl green.

Safranin, strong aqueous solution: membranes not stained, cyst contents freely stained.

Gentian violet, strong aqueous solution: membrane not stained, cyst contents stained.

Eosin, strong aqueous solution: membranes not stained, cyst contents stained.

Carbol fuchsin, the strong bacteria stain: membranes not stained, contents of endocyst stained deep red.

Hæmatoxylin (Heidenhain's): film-preparations made by hatching out *Colpoda* in 1-per-cent. hay-infusion + egg white and killing the excysting forms with osmic vapour, were stained with iron hæmatoxylin. The ectocysts stained dark blue or purple; the endocysts stained the same tint but not so intensely. Hæmatoxylin (Delafield's): in films prepared as for Heidenhain's, but stained with this preparation, the ectocysts stained purple, the endocysts a pale reddish or bluish purple. From these records it will be seen that only the hæmatoxylin stains touch the cyst-membranes. This is interesting, for Fabre considered that many aniline stains affected the cyst-membranes of ciliates.

The Nature of the Process of Excystation.—Bütschli* says that little is known on the manner in which the cyst-contents are reorganised prior to excystation. No doubt water penetrates into the interior causing the organism to swell up and the contractile vacuole to begin pulsating. The cilia reorganise themselves, but how they do so is not accurately known. The imbibition of water doubtless plays an important part in the rupturing of the ectocyst.

He also suggests that instead of *Colpoda* escaping from the temporary division-cysts through the narrow aperture, they may perhaps have the power of dissolving the membrane in a particular place.

Many observers have recorded the fact that the ectocyst is caused to rupture, as Bütschli suggests, by the increased volume of the cyst contents owing to the imbibition of water. The exact manner, however, in which the organism manages to get out of the endocyst does not appear to have been determined. It is on this interesting point that the present investigation throws light.

In carefully watching the movements of *Colpoda* within the transparent endocyst, it is noticed that the organism rotates freely and that the endocyst gradually increases in diameter; the wall becomes thinner and thinner until, finally, it becomes invisible, at which time the *Colpoda* swims away. The whole process only occupies from 5 to 10 minutes, when incubation has taken place at 30° C. It seems as if the endocyst is gradually dissolved or digested so that the enclosed organism may be liberated.

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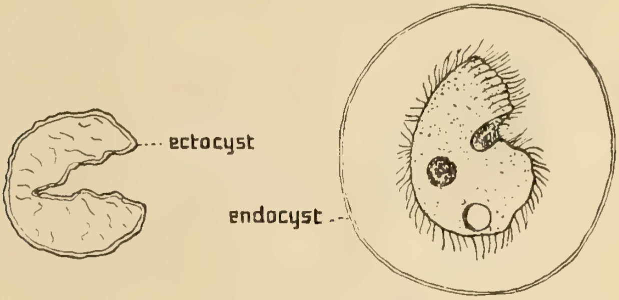
* Bütschli, O., 'Bronn's Klassen des Thierreichs,' "Protozoa," Abt. III, p. 1664.



In order to determine as far as possible the nature of the process, the following methods were adopted :—

Killing Excysting Colpoda with Different Reagents.—Hanging-drop cultures were made in the usual way, and when there were several *Colpoda* rotating within their endocysts the cover-slips were lifted and the cultures exposed for a short time to the action of the vapour of some particular reagent capable of killing the organisms already liberated and those still excysting. The cover-slips were then waxed to the slides again. When volatile antiseptics were used, such as xylene, toluene, chloroform, and carbon disulphide, the organisms were killed within 30 seconds, and then it was observed that the endocyst still continued to increase in diameter, the wall becoming gradually thinner and thinner until finally it disappeared.

This was determined accurately by taking measurements of the endocyst at different times with an eye-piece micrometer. In some cases the endocyst disappeared in the course of 10 to 15 minutes, and in other cases after the lapse of a few hours.

When osmic vapour and vapour of 40 per cent. formalin were used the *Colpoda* were killed in a few seconds. It was then noticed that the endocysts did not continue to swell up and disappear, but remained exactly as they were, in diameter of cyst and in thickness of wall, at the moment of killing. There was no alteration even after 24 hours.

This interesting difference between the effect of volatile antiseptics and osmic acid and formalin vapour indicates that the process of excystation from the endocyst is normally effected by the secretion of a solvent or digestive ferment. The antiseptics, toluene, chloroform, etc., kill the excysting organisms but not the enzyme which it is secreting, and thus the process of endocyst-digestion continues. On the other hand, formalin and osmic acid vapour kill the organism and also the secreted enzyme, hence the digestion of the endocyst is stopped.

After the organism has been killed by a volatile antiseptic the further digestion of the endocyst is not so rapid as when the organism is actively swimming about within, but this may be due to the fact that normally an excysting *Colpoda* has a considerable mechanical effect upon the thin endocyst wall as it swims about, no doubt aiding in its more rapid destruction.

Tests on Soluble Starch.—In order to test the effect of the enzyme secreted by *Colpoda* on soluble starch an agar medium was made in the following proportions—100 c.c. 0·05-per-cent. solution of soluble starch, agar-agar 0·8 grm. Two sterile Petri dishes were poured with this medium, and each was inoculated with cysts of *Colpoda* which had been well teased out in sterile distilled water. There were ten or twelve areas of inoculation on each plate, each about 3 mm. in diameter.

After incubating at 30° C. for an hour and a quarter, active and excysting *Colpoda* were found on many of the inoculated areas. One or two *Colpoda* were seen to emerge from their digested endocysts, and at these spots traces of iodine in potassium iodide solution were added. A uniform blue coloration resulted over the whole of the region; there were no clear uncoloured zones in the region of the digested endocysts. As controls, traces of the iodine in potassium iodide solution were added in close proximity to the inoculated areas. These gave exactly the same tone of blue as in the areas where *Colpoda* had excysted.

It is evident therefore that the enzyme secreted by *Colpoda* during excystation is not capable of digesting soluble starch.

Tests for the Digestibility of the Endocyst by Different Ferments.—Having determined that the endocyst is digested by enzymic activity, it was deemed advisable to test the digestive powers of pepsin and trypsin upon it. Hanging-drop cultures were made in the usual way with resting cysts and the excysting organisms were killed at the proper moment with osmic acid vapour. Two or three cultures were then supplied with a platinum loop or two of 0·04-per-cent. pepsin in 0·2-per-cent. hydrochloric acid,* and two or three supplied with an equal quantity of 0·04-per-cent. trypsin in 0·5-per-cent. caustic soda solution. All the cultures were then waxed down again and put into the incubator at 40° C.

Examinations were made at different times to ascertain the effect of the ferment, but in no case was the endocyst digested either by pepsin or trypsin even after remaining for several days at 40° C. Pepsin had the effect of turning the *Colpoda* almost black, whether they were free or enclosed within the endocysts.

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* The pepsin and trypsin were made at this strength according to the instructions in Hoppe-Seyler's 'Handbuch der Physiol. und Pathol. Chemisch. Analyse,' 7th Edit., 1903.

Trypsin on the other hand gradually digested the *Colpoda* already free and those within the endocysts, rendering them very transparent and causing complete solution and digestion of all the organism except certain very refractive granules. These two digestive ferments were tried on many occasions but at no time were they found to attack the endocyst.

Diastase.—The diastase used was prepared from fresh pale-barley malt by the method described by O'Sullivan.* According to Brown and Escombe,† diastase acts best in very dilute acid solutions and they recommend 0·006-per-cent. formic acid. The diastase used was therefore dissolved in formic acid of this strength. One lot was heated up in a water bath at 62–65° C. for about 20 minutes in order to kill off any cytase which might be present.

Two solutions of diastase were thus obtained, one heated and the other unheated. Hanging-drop cultures were made and the excysting *Colpoda* killed with osmic vapour. One or two platinum loops of the diastase solutions were added and then the cover-slips were waxed down again and the culture put into the incubator at 40° C.

Twenty-four hours after the addition of the diastase no endocysts could be found even after careful search; they had all been digested, both by the heated and the unheated diastase. Similar results were obtained by repeating the experiment on other occasions.

Ptyalin.—Saliva was collected and diluted slightly with distilled water. It was then filtered and a small crystal or two of thymol added to prevent putrefaction. A platinum loop or two of the filtered liquid was added to hanging-drop cultures, which were then allowed to incubate at 40° C. When perfectly fresh saliva was used the endocysts were digested somewhat slowly, *i.e.* in 48 hours.

Some of the cultures made did not show the digestive action, whilst others did. It is possible that a certain amount of change must first be effected by the enzyme secreted by the *Colpoda* within, before ptyalin can act on the endocyst. The digestive powers of this ferment on the endocyst are therefore uncertain.

From these experiments it is evident that the endocyst consists of a substance which is not digested by pepsin or trypsin, but which is completely digested by diastase and in some cases by ptyalin. It is therefore highly probable that this endocyst material is of a carbohydrate nature. This raises a point of considerable interest, for the endocyst was digested by the heated as well as by the unheated diastase, thus showing that the seat of the enzymic activity was not confined to any cytase which might have been present in the diastase as an impurity.*

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* O'Sullivan, C., 'Trans. Chem. Soc.,' 1884, vol. 45, p. 2.

† Brown and Escombe, "On the Depletion of *Hordeum vulgare* during Germination," 'Proc. Roy. Soc.,' 1898, vol. 63.

Diastase is generally considered to act only upon starch and glycogen. The substance under consideration is certainly neither starch nor glycogen and yet it is digested by diastase. In this respect also it differs from paramylum, which is untouched by diastase.

Paraglycogen is soluble in hot water, and the solution is hydrolysed into dextrin and a trace of reducing sugar, thus differing from glycogen, which yields dextrin and plenty of reducing sugar when hydrolysed with the salivary ferment. It is because of this difference that Bütschli† called it paraglycogen.

Since diastase digested the endocyst, chemical tests for carbohydrates were sought. Attempts were made to obtain an osazone from the liquid in which *Colpoda* had been caused to excyst, after proper treatment with sodium acetate and phenyl-hydrazine hydrochloride solutions, but only negative results were obtained. It was concluded that the carbohydrate produced by the digestion of the endocyst was present in far too small a quantity to be detected by this method.

Colour Reactions for the Detection of Carbohydrates.—A method‡ was found by which dextrose, lactose, saccharose, starch, and cellulose, when heated up with strong hydrochloric acid and scatole, yield a violet coloration, the reaction still showing at a dilution of 1 : 300,000. Fifteen grains of scatole were obtained, and tests were made with dextrose, starch, lactose, saccharose, cellulose, and hay-infusion, by heating them in test-tubes with strong hydrochloric acid and small quantities of scatole. In all cases a violet coloration of the liquid resulted, even when dextrose diluted 1 : 300,000 was used. Hay-infusion could not, therefore, be used for the excystation of *Colpoda* from cysts in this experiment.

Cultures were therefore made in distilled water containing 0·01-per-cent. caustic soda, this being the percentage of alkali in the hay-infusion commonly used. The object of the experiment was to obtain a colour reaction for the presence of a carbohydrate in the culture-liquid containing the products of the digestion of the endocysts by the enzyme secreted by the *Colpoda.*

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* Occasionally one or two fibres of cellulose from the filter paper on which the cysts had been collected were introduced into the hanging drops along with the cysts. These fibres never showed signs of corrosion or solution, thus showing the freedom of the diastase from cytase.

† *Loc. cit.*, p. 1484.

‡ 'Jour. Chem. Soc.,' 1907, vol. 92, Pt. 2, Abst., p. 308 ("Colour Reactions of Carbohydrates with Indole and Scatole").

The cultures were made in the hollows of cavity slides, and the cysts of the large variety of *Colpoda cucullus* were used. After a large number of organisms had become active, the cultures were unsealed, and the liquid was taken up in a capillary pipette and transferred to a small glass tube. This liquid was practically free from ectocyst membranes, though it is possible there may have been a few present, for it was impossible to examine the liquid under the microscope. There may also have been one or two fine threads of cellulose present from the filter paper on which the cysts had been collected from the original culture of *Colpoda.*

To the liquid was added an equal volume of strong hydrochloric acid, and the mixture was boiled for half an hour on the water-bath, in order to hydrolyse any maltose present to dextrose. A little scatole was added and a drop or two more of strong hydrochloric acid. On gently heating this mixture over the Bunsen flame a pale purple coloration resulted, indicating the presence of a carbohydrate in the liquid.

On heating up some of the original culture-liquid, *i.e.* distilled water containing 0·01 per cent. caustic soda, with strong hydrochloric acid and scatole, no coloration was obtained, nor was any coloration produced when distilled water alone was tested in this way. The liquid, then, which had been used for the excystation of *Colpoda* clearly contained a carbohydrate. That the carbohydrate was there as a product of the digestion of the endocyst of *Colpoda* cannot be definitely asserted, since there may have been present in the liquid a trace of cellulose, or ectocyst membrane. Whether this supposed cellulose and ectocyst would be sufficient to account for the coloration produced cannot be stated, but I consider it negligible, and think it does not vitiate the result of the test.

No special emphasis, however, is laid on this test as an indication of the carbohydrate character of the endocyst. The fact that it is digested by diastase and fails to show any reactions to protein and other tests is sufficient to show that it is a true carbohydrate.

Note on the Endocyst of Gastrostyla steinii.

A few cultures were made with the resting cysts of *Gastrostyla steinii,* and several active organisms were found after three hours' incubation at 30° C. There appear to be ectocysts and endocysts in the resting cysts of this organism as in *Colpoda.*

The process of excystation is slower than in *Colpoda cucullus,* and many organisms appear to rotate and cause digestion of the endocyst whilst still within the confines of the ectocyst, afterwards making their exit from the latter by forcing their way through a rupture in the wall.

Some free endocysts were tested with iodine in potassium iodide solution but they did not stain, nor was any alteration in colour or shape effected by the addition of 70-per-cent. sulphuric acid to the preparation. This indicates that the endocyst of *Gastrostyla steinii* is in all probability of the same nature as that of *Colpoda cucullus.*

Note on the Initial Stages of Encystation in Colpoda cucullus.

On examining a hanging-drop culture in which *Colpoda* had been active for two days many organisms were found to be encysting, probably commencing to form resting cysts, since there was not an abundance of food. An opportunity therefore presented itself for the study of the earliest stages of encystation.

Whilst examining under the oil-immersion a group of three rounded *Colpoda* which were revolving, each within a limiting membrane, a free-swimming *Colpoda* came into the field. It did not move away but began to revolve, the cilia moved very violently, and the organism began to lose its characteristic form and outline and became more rounded. It appeared as though in this initial stage of encystation some jelly-like substance or mucilage were exuded from the ectoplasm, and that because of this the cilia had great difficulty in moving. They seemed to move in bunches which gave the appearance of waves on the surface of the organism. Shortly after this there was a definite limiting membrane within which the organism was revolving, clearly propelled by means of its cilia, which were easily visible under the oil-immersion.

This is in accord with Rhumbler's observations. He watched the formation of the cyst membranes in division cysts and resting cysts of *Colpoda,* and was convinced that rotation within the cyst was due to ciliary activity.

Summary and Conclusions.

1. The cyst membranes of *Colpoda cucullus* consist of the outer ectocyst and the inner endocyst. The ectocyst is insoluble in strong acids, gives no reaction with iodine and strong sulphuric acid, only stains with hæmatoxylin, is insoluble in alcohol and ether, and is only dissolved by 20-per-cent. caustic soda. In all these properties except the absence of any reaction to iodine, it resembles the outer cyst wall of *Euglena viridis,* investigated by Bütschli, which is composed of a nitrogen-free carbohydrate compound.

2. The endocyst is composed of a transparent substance which is insoluble

in cold or hot water, strong acids, fat solvents like alcohol and ether. It is soluble in 4-per-cent. caustic soda at 30° C., and fails to give any reaction with iodine and strong sulphuric acid. It is not digested by pepsin or trypsin but is completely digested by diastase acting at 40° C., and is sometimes slowly digested by the salivary ferment, ptyalin, also at 40° C.

It is thus of a carbohydrate nature but differs from all other carbohydrates which have been found in the protoplasm or secreted by the protoplasm of protozoa. It seems to consist of a substance allied to glycogen, paraglycogen and paramylum. The name Cystose is proposed for it. There is evidence to show that the endocyst of *Gastrostyla steinii* is composed of a similar substance.

3. During the normal process of excystation the endocyst is set free by the rupture of the ectocyst and the *Colpoda* liberates itself by the rapid digestion of the endocyst by means of a powerful enzyme which it secretes. This enzyme is put out of action by killing the excysting organisms with formalin or osmic acid vapour, but continues to act on the endocyst when the organisms are killed with toluene, ether, chloroform or carbon bisulphide vapours. It can act when the medium surrounding the cyst contains 0·19 per cent. of caustic soda on the one hand and 0·09 per cent. of hydrochloric acid on the other, and may therefore be said to act in alkaline, neutral and acid media.

It cannot act at 40° C., since excystation is inhibited at this temperature, but it appears to act best at 30° C. In these respects it is remarkable and differs strikingly from diastase, the activity of which is retained even as high as 68° C., but is entirely checked in the presence of less than 0·04 per cent. of caustic soda. A further point of difference from diastase is found in the fact that the new enzyme fails to digest soluble starch. The name Cystase is proposed for it.

General Conclusions.—The endocyst membrane of *Colpoda*, and probably of other ciliated protozoa forming double-walled resting cysts, is composed of a carbohydrate which is different from all carbohydrates previously described. To this substance the name "Cystose" is given. During the process of excystation the endocyst is digested by a powerful enzyme secreted by the enclosed *Colpoda*. The name "Cystase" is proposed for this ferment.

My best thanks are due to the Lawes Agricultural Trust for allowing me to carry on the work at this Laboratory. I am also indebted to various members of the Laboratory staff and other friends for helpful suggestions, particularly to Mr. W. A. Davis. Organic Chemist here, for suggesting the names "Cystose" and "Cystase."

Protostigmata in Ascidians.

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(Communicated by Prof. A. B. Macallum, F.R.S. Received March 3,—Read April 24, 1913.)

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Introduction.

It has been shown that at a certain stage in the ontogeny of Ascidians there is usually a definite number of stigmata or gill slits, which are elongated dorsoventrally and arranged in a longitudinal series on each side of the pharynx. To these the name protostigmata was given by Garstang in 1892. The stigmata of the adult differ from these in being usually very numerous, indefinite in number and elongated antero-posteriorly.

In 1904 Julin and Damas made two separate proposals to base the classification of the Tunicates on the condition of the protostigmata. As these proposals differed rather widely from each other and from the current classifications based upon the adult condition, I have taken up the question as far as it concerns the Ascidians and have investigated the origin of the stigmata in a large number of genera. I have been able to confirm for myself very many of the observations that have been published regarding the origin of the stigmata in the genera *Holozoa* [*Distaplia*], *Clavelina, Ciona, Corella, Cæsira* [*Molgula*], *Botryllus* and *Dendrodoa* (*Styelopsis*). In addition I have investigated the genera *Amaroucium, Polycitor* [*Distoma*], *Sycozoa* [*Colella*], *Ascidiopsis* (very near *Ascidiella* and *Phallusia*, which have been studied by others), *Chelyosoma, Styela, Boltenia, Pyura* [*Cynthia* pars] and *Tethyum* [*Cynthia* pars]. The last three genera belong to the family Tethyidæ [Cynthiidæ], no members of which have been previously investigated as to the origin of the stigmata.

My interpretation of the course of development in these genera tends to show that the protostigmatic condition supports the classification advanced by Seeliger and revised by Hartmeyer.*

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* Bronn's 'Kl. u. Ordn. des Tierreichs.'

Grades of Protostigmata.

In fig. 1, C represents diagrammatically the left side of the pharynx of a young *Ciona*. It can be clearly seen that there are two grades of protostigmata. Counting from the anterior end (left side) the first, fourth, and fifth are longer than the second, third, and sixth. The former are the first to appear and they perforate the pharyngeal wall independently of each other. They give rise to the latter three as indicated in the figure, by their ventral ends extending and turning upward. They then divide at the angles. De Selys and Damas (1901) have proposed the term primary protostigmata for the three arising by independent perforation and the term secondary protostigmata for the six resulting from their division.

But the fourth protostigma differs from the first and fifth in having its concave face forward and in turning forward and upward before division instead of backward and upward. Willey (1893), De Selys (1901) and Damas (1904) have recorded instances in *Ciona* and *Ascidiella*, in which the first and fourth protostigmata were continuous ventrally (before the second and third had been formed). Willey concluded from this that the first four protostigmata were derived from a single primary gill slit. This view seems to be justified by the facts. The fourth behaves as if it had been derived from the first in the same way that the sixth is derived from the fifth. The fourth is equal in size to the first because it is derived from the latter at such an early stage, before perforation. They are both able to repeat the process and thus form four stigmata before the change occurs that brings about the formation of the definitive stigmata. The fifth is able to divide once only.

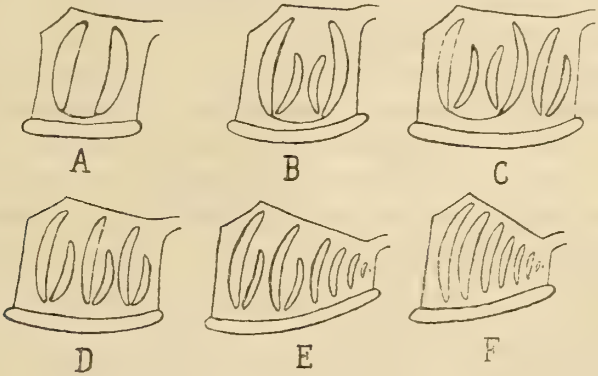

FIG. 1.—Various types of protostigmatic condition. The left side of the pharynx is shown, the anterior end being toward the left.

I conclude therefore that in *Ciona* the stigmatic rudiment or *anlage* divides into two parts placed one behind the other. They correspond in position to the first and fifth. These may be called the protostigmata of the first order or primary, using the term proposed by de Selys and Damas but in a somewhat different sense. They correspond to a certain extent with the "primary gill slits" of Willey and the "fentes branchiales" of Julin (1904). This early division of the stigmatic rudiment produces elements with a similar orientation. When they elongate to form the protostigmata, their concave surfaces are posterior.

The subdivision of the two primary protostigmata produces four protostigmata of the second order or secondary. They correspond in position with the first, fourth, fifth, and sixth. The two members of each pair are oriented differently, one being the reverse of the other.

The process of subdivision is repeated in the case of the first pair, giving four protostigmata of the third order or tertiary, namely, the first, second, third and fourth. Here also of each pair one is the reverse of the other. There are no Ascidians in which there has been shown to be further subdivision to form new protostigmata.

The protostigmata are formed in two ways. First, by simple subdivision; second, by modified subdivision, resulting in the intercalation of new stigmata. By these same processes acting in the same order, the protostigmata are converted into the rows of definitive stigmata of the adult. Frequently the two processes are not altogether distinct. An intermediate method of subdivision may occur.

The Protostigmatic Condition in the Various Groups.

Julin and Damas have blazed the way for a comparison of the various kinds of stigmatogenesis to be found in Ascidians. Both, however, have based their classifications on definite stages, and have more or less ignored the differences in the process.

Damas (1904) uses as a basis the final protostigmatic condition, and divides the Tunicata into the Mono-, Di-, Tetra-, Hexa- and Poly-prostigmata. The Cæsiridæ [Molgulidæ] are separated from the Tethyidæ [Cynthiidæ], Styelidæ, etc., and placed with the Cionidæ, Phallusiidæ, etc. And, as I shall show subsequently, some of the Tethyidæ would have to be placed in the Hexa- and others in the Poly-prostigmata. Damas also places the Pyrosomatidæ and Doliolidæ with the Tethyidæ, etc. These are unnatural groupings.

Julin (1904*a*) uses as a basis the number of stigmata that perforate independently and calls them "fentes branchiales." He makes three distinct groups: those with one pair of "fentes branchiales," those with two pairs, and those with three pairs. To the first group belong the pelagic Tunicata, Appendicularians, Salpidæ, Doliolidæ and Pyrosomatidæ ; in the second group are the Krikobranchia and the Perophoridæ ; and in the third group all the simple Ascidians. These groups are natural enough, but I cannot agree with his interpretation of the development in many forms. Having to prove that all the stigmata of the adult are derived from a limited number of first stigmata, he puts a rather forced construction on many of the facts. If his method were followed out fully, all the stigmata in each and every Tunicate could be derived from a single pair of stigmata. I think that this is a legitimate conception, but it does not lead to any divisions within the group. The Appendicularian is without doubt a primitive form. The Doliolidæ, Pyrosomatidæ and Ascidiaceæ show remarkable diversity in the methods by which a large number of stigmata are derived from a single pair corresponding with those of the Appendicularian. It is by studying these methods that we get an insight into the affinities of the various forms. Mere numbers at any definite stage are of subordinate importance to the process.

The rather definite separation of the process into two parts, (1) the formation of a longitudinal series of protostigmata, and (2) the transformation of these into transverse rows, makes it possible to institute a comparison of the early stages alone. In fig. 1 I have represented diagrammatically various types of protostigmatic condition, indicating not only the number but also the method of origin.

A represents the condition in the Krikobranchia, a group consisting of the majority of the compound Ascidians. I agree with Julin and Damas in considering that two protostigmata are represented in this group. True protostigmata are never seen. The process of division into definite stigmata begins before perforation, as will be shown subsequently. The two protostigmata are evidently of the second order, and represent a single primary protostigma.

B represents the condition in *Perophora*, modified from the description by Damas. Four protostigmata of the third order are present, representing a single primary one. Here also typical protostigmata do not occur, and the interpretation is doubtful, as will be shown subsequently.

C represents the condition in other Dictyobranchiates (*Ciona*, *Phallusia*, etc.). This condition has been described above. There are four tertiary protostigmata, representing a single primary, and behind these two secondary ones, representing another primary. These forms are peculiar in possessing two primary protostigmata.

D shows the condition in the Cæsiridæ and some Tethyidæ (*Pyura* and *Tethyum* [*Cynthia*]). There are six secondary, representing three primary protostigmata.

In E is shown the condition in some Tethyidæ (*Boltenia*) and some Styelidæ (*Styela*). There are four secondary, followed by an indefinite number of primary.

F represents the condition in some Styelidæ (*Dendrodoa* (*Styelopsis*)) and in the Botryllidæ. There are an indefinite number of primary and no secondary protostigmata. Julin has given an entirely different account of the origin of the protostigmata in *Dendrodoa* (*Styelopsis*) from that given by Damas. My investigation of this genus is in accord with the account given by Damas. It seems possible that Julin's material has been wrongly identified as belonging to this genus.

Damas (with Garstang and Seeliger) considers the single row of stigmata in the Pyrosomatidæ and Doliolidæ as being a series of protostigmata. Julin (with Lahille) considers the row as homologous with one of the transverse rows of stigmata in an Ascidian. I favour the latter view. Damas' objection that the longitudinal bars of *Pyrosoma* would, in that case, not be homologous with those of Ascidians is valid, but they are readily homologised with the internal transverse vessels of *Holozoa* and of many simple Ascidians, as done by Lahille (1890).

The series of fig. 1 shows gradation in two respects. There is a continuous increase in the number of primary protostigmata, and, excepting A, there is a continuous decrease in the ability of the protostigmata to subdivide, this decrease being greater posteriorly than anteriorly. The variation shown in the families Tethyidæ and Styelidæ is noteworthy. The former family contains some forms with a limited number (three) and others with an indefinite number of protostigmata. Up to the present, a relatively small proportion of the existing genera have been investigated. Further studies will doubtless show even greater variations.

The Factors of Stigmatogenesis.

In interpreting the various stages that may be examined, it is important to keep in view the factors involved in the process. The Ascidian pharynx offers a splendid field for a study of the processes of development, since great variations are shown in the different groups, and the processes are practically confined to two dimensions, *i.e.* one plane.

As the basis of the process we have, of course, the properties of the cells and their interaction with each other and with the surrounding water. These determine the ways in which the cells divide and arrange themselves to give the gross characters that are seen.

Certain gross factors (depending upon the above), which vary in the different groups, may be mentioned.

1. *Growth.*—This takes place chiefly at the ends of the stigmata, and results in elongation.

2. *Perforation.*—This is the appearance of an opening in a fused area of the pharyngeal and atrial walls. It may occur early or late.

3. *Subdivision.*—This is the division of a stigma by the growth across it of a bridge. It may occur early or late in the growth of a stigma; it may occur at a varying point on the stigma; it may occur before or after perforation.

4. *Orientation* or *Direction of Growth.*—This factor determines the shapes and the positions of the stigmata with reference to each other. It depends upon equality or inequality of growth of the cells on either side of a stigma. Great variation is shown in this factor.

By variations in these four factors there results the infinite diversity in the stigmata of Ascidians. A comparatively slight variation may make a great difference in the final picture, and a similar end result may be attained by following quite different paths. One should avoid giving too much significance to certain differences shown. It is certainly astonishing to see, in a young colony of *Botryllus,* the first blastozoid provided with four rows of minute definitive stigmata that have arisen by independent perforation, and the oozoid beside it with six large protostigmata that never become converted into definitive stigmata.

The Origin of the Stigmata in the Various Groups.

In fig. 2 the methods of development of the protostigmata in the various groups are schematically represented. They all are seen to be derived from a common starting point, a single stigma or rudiment not yet perforated. The stigmata represented in solid black are those that have not yet perforated. Many of them are hypothetical. Usually a dark mass can be seen at the point where a stigma will shortly perforate.

Perhaps the simplest method is the fourth of the figure, that occurring in the Botryllidæ and some Styelidæ. The protostigmata appear in order beginning in front. Each stigma is formed by independent perforation at a point behind the middle of the previously formed one. Julin (1904*a*) has maintained that in *Dendrodoa* (*Styelopsis*) *grossularia* the protostigmata arise in a manner analogous to that of *Ciona* described above. Both Damas (1904) and Fechner (1907) describe their origin by independent perforation and give excellent figures. In an allied species (*Dendrodoa carnea*) I have found no indication of intercalation or of new protostigmata being formed from pre-existing ones, the conditions being the same as figured by Damas and Fechner.

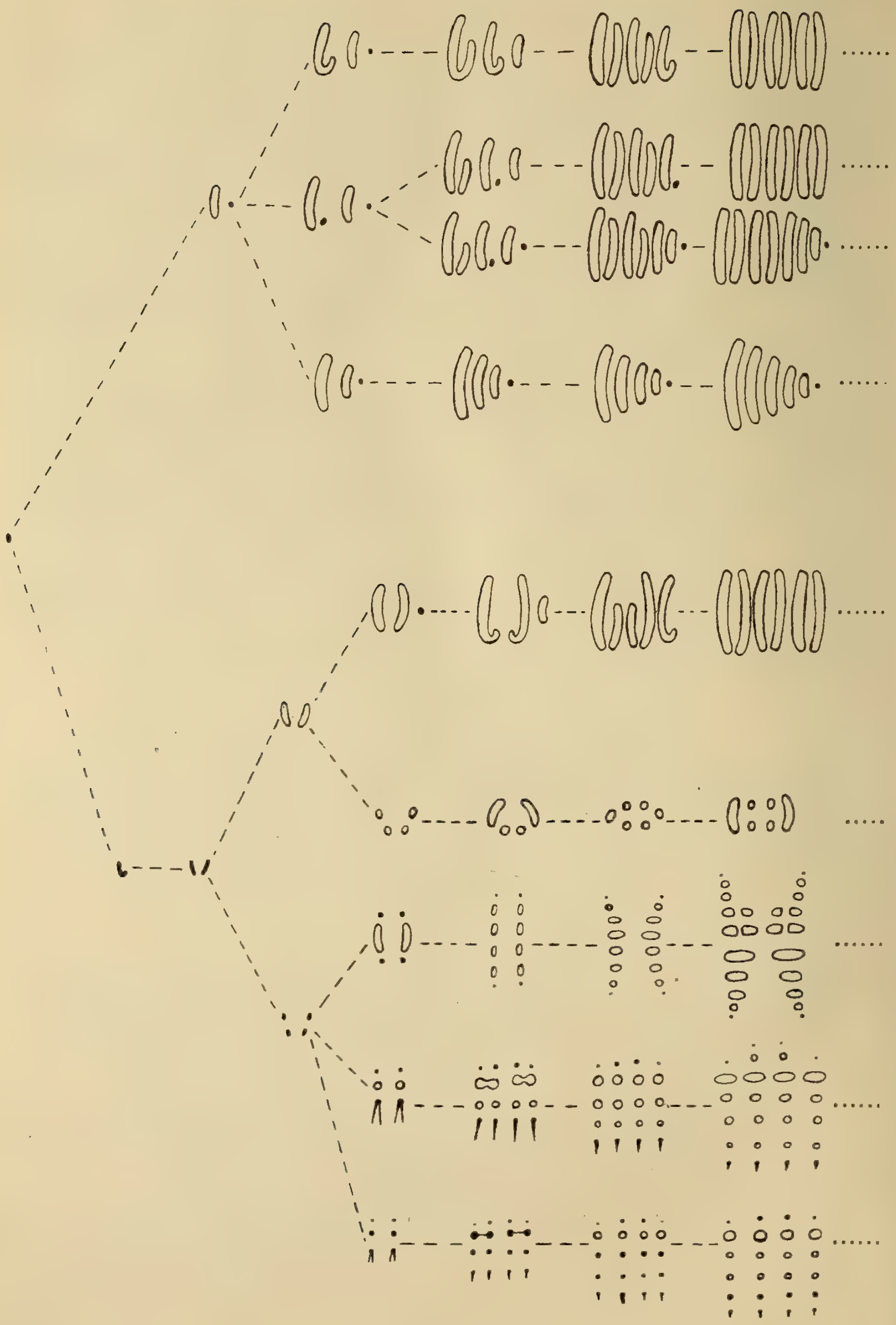

Fig. 2.—Schematic representation of variations in early stages of development of stigmata in

……………………	*Cæsira*	Cæsiridæ	Ptychobranchia.
	Eugyra		
	Pyura	Tethyidæ	
……………………	*Tethyum*		
……………………	*Boltenia*		
	Styela	Styelidæ	
……………………	*Dendrodoa*		
	Botryllus	Botryllidæ	
……………………	*Chelyosoma*	Chelyosomatidæ	Dictyobranchia.
	Corella		
	Ascidiopsis	Phallusiidæ	
	Ascidiella		
	Phallusia		
	Phallusiopsis		
	Ciona	Cionidæ	
……………………	*Perophora*	Perophoridæ	
……………………	*Clavelina*	Clavelinidæ	Krikobranchia.
	(*Archiascidia*)		
……………………	*Holozoa*	Polycitoridæ	
	Cystodites		
	Sycozoa		
	(*Polycitor*)		
……………………	*Polycitor*		
	Amaroucium	Synoicidæ	

Ascidians. The stigmata of the left side are shown, the anterior end being toward the left.

The number of protostigmata is indefinite. Damas gives more than 15, Julin 12 at least. In the Botryllidæ the number is smaller; from 5 to 7 have been observed. Their method of formation has not been established. The majority appear about the time of metamorphosis, when observation is difficult. I can affirm independent perforation for the sixth in a species of *Botryllus* from Naples. The others in size show no evidence of intercalation.

In the Cæsiridæ we have in many respects the other extreme. The number is definite and intercalation is regular. Three stigmata perforate in the usual order and each one by turning upward at the ventral end and dividing gives rise to a second placed immediately behind it, as is shown in the first series of stages in the figure. Six protostigmata arranged in three pairs are thus formed. The same method is followed by *Pyura haustor,* one of the Tethyidæ. I have observed in this species the derivation of the second and fourth from the first and third, but not of the sixth from the fifth. Only six protostigmata are formed and their first subdivision to form the definitive stigmata is the same as that which has been described for the Cæsiridæ.

A slight modification of this method is to be found in another Tethyid, *Tethyum* [*Cynthia*] *pyriforme.* The turned up portion of the stigma is cut off very early, probably before the opening of the stigma has extended into it. I have not observed all the stages but can affirm that the second is intercalated from the first and that only six protostigmata are formed. Also in the earliest stage examined only one stigma is present.

A further modification occurs in the genera *Boltenia* (*B. ovifera* and *B. villosa*) and *Styela* (*S. gibbsii*) of which I have examined a full series of stages. The second and fourth are formed by the abstraction of the ventral growing tips of the first and third. Subsequently these perforate and extend dorsoventrally. The fifth and all succeeding protostigmata arise in order by independent perforation at points along the middle of the side of the pharynx. They give no indication of being derived from pre-existing ones. Their number is indefinite. I have observed stages with 11 in *Styela* and with 9 in *Boltenia.* The two genera differ greatly in the method by which the definitive stigmata are formed from the protostigmata.

The method just described for these two genera is an approach to the method of *Dendrodoa* and the Botryllidæ. The third primary protostigma has lost its ability to form a new one by intercalation. If the first and second likewise lost that power we would have the condition in *Dendrodoa.*

All the forms so far considered belong to the Order Ptychobranchia. This group is therefore characterised by having three or more primary protostigmata, which perforate in regular order. Secondary protostigmata may or may not be formed and are never more numerous than six. This Order is sharply marked off from the other two Orders.

Inside the Order, the four methods of formation of protostigmata do not correspond with the four families of the Order. The Tethyids exhibit three methods and the Styelids two. But comparative anatomy has shown that some of the Tethyidæ approach the Cæsiridæ and others approach the Styelidæ. And in a similar way the Styelidæ show affinities with both the Tethyidæ and Botryllidæ. The protostigmatic condition therefore corroborates the classification based upon the adult condition.

There are two distinct methods in the Dictyobranchia. The most general one is that described above for *Ciona.* The first stage is the appearance of two stigmata of about the same size. From their occasional connection with each other and from their subsequent behaviour, they are very evidently halves of an original single element which is doubtless homologous with the first stigma of the Ptychobranchiate. They are secondary protostigmata, corresponding with one primary. Their probable history before perforation is indicated in the figure. The original element has turned up at its lower end in the usual fashion and then divided into two.

The two secondary protostigmata subdivide into four tertiary, and at the same time a second primary appears and divides into two secondary ones. The new stigmata are connected for a short time with the old in *Ciona*, and this has been shown in the figure. In *Ascidiopsis* (and doubtless in other genera as well) the intercalated stigmata are formed as has been described above for *Styela* and *Boltenia.* Stigmatic material is separated from the lower ends of the old stigmata, and subsequently cavities appear in the new parts. This is only a slight modification of the process occurring in *Ciona.*

In *Perophora* the accounts that have been given do not agree. Julin states that the stigmata arise essentially in the same way as in *Distaplia*, but gives no figures. Damas has figured the process and interprets it in such a way as to bring it into line with the condition in other Dictyobranchiates. Not having an opportunity of examining this genus, I have followed Damas' account. The figures for this genus given in the scheme of fig. 2 are from Damas. The two stigmata that are the first to appear evidently correspond with the similar two of other Dictyobranchiates, that is, they are two secondary protostigmata representing a single primary. By division and intercalation four tertiary are formed. The process is repeated dorsally as shown in the figure. As a result of this curious repetition the second and third protostigmata are each represented by a dorsal and a ventral part derived from the dorsal and ventral ends of the first and fourth. Damas has compared the pharynx of *Perophora* with the anterior two-thirds of the pharynx of *Ciona*.

The Perophoridæ differ, therefore, from the other Dictyobranchiates in having only one primary protostigma instead of two. They are also peculiar as to the method of origin of the second and third protostigmata or protostigmatic rows as they should be called. It may be that this method is to be compared with that in *Holozoa* [*Distaplia*] as maintained by Julin.

In the Krikobranchiates the process is much modified by the division into definitive stigmata occurring before or at the time of perforation of the protostigmata. This is foreshadowed in *Perophora*. In *Clavelina* the first stage is that with two stigmata, which appear simultaneously. The manner in which these stigmata are curved and also their subsequent behaviour show that one is the reverse of the other. They are evidently homologous with the two first stigmata of the Dictyobranchiate, as has been argued by Julin and Damas. They are secondary protostigmata, representing one primary. By equal subdivision and also by budding at the ends (these buds perforate after separation) two rows of stigmata are formed. This is the division into definitive stigmata. There is a tendency for this to occur before perforation of the protostigmata. By a transverse division of all the stigmata of a row the number of rows is doubled. The beginning of this process is shown for *Clavelina* in the last stage in the figure. This is the usual Ascidian method for increasing the number of rows during the later stages of development. It occurs relatively early here, and we shall see that it occurs still earlier in other Krikobranchiates.

In the figured scheme of the development in *Clavelina* the hypothetical processes occurring before perforation are the same as for the Dictyobranchiate with the addition of the tendency of the protostigmata to divide into definitive stigmata before perforation. In *Archiascidia*, according to Julin (1904*b*), the development of the stigmata is essentially the same as in *Clavelina*. It differs from the latter in the failure of the two primary rows of stigmata to divide.

Of the remaining Krikobranchiates that have been studied, *Holozoa* [*Distaplia*] shows the simplest condition. This has been represented in the lowest but one series of stages in the figure. Two stigmata appear simultaneously. They are evidently homologous with the two first in *Clavelina*, although there is no very definite indication of one being the reverse of the other. They are to be considered as two secondary protostigmata representing one primary. They elongate and divide dorsoventrally, and about the same time four new stigmata perforate just below. By new perforations on the ventral side, and at a later stage on the dorsal side as well, four transverse rows of stigmata are formed. These are the definitive stigmata. Not only have the protostigmata divided into definitive stigmata before perforation, but also the doubling of the number of rows by a parallel division of all the stigmata in each row has been nearly completed before perforation. Only one stigma of each row (the first one to appear) is still undivided at perforation. A species of *Sycozoa* [*Colella*] that I have been able to examine shows a slightly more advanced condition. Usually the first stigma of each row to appear is nearly or altogether divided before perforation, the apertures of the stigmata resulting from the division not connecting with each other.

Daumezon (1909) states that in *Cystodites* the process seems to be the same as in *Holozoa*, but that in *Polycitor* (*Eudistoma*) the second of the two first stigmata to appear does not divide, and as a result only three rows are formed instead of four. The adult and larva of his species show an imperforate area in the posterior quarter of the pharynx corresponding to the absent fourth row. In a Pacific species of *Polycitor* (*Eudistoma*) with three rows of stigmata, but, as far as I can see, without any very definite imperforate area, only three rows of stigmata are formed in the larva. The pharyngo-atrial wall projects into the pharynx in the form of a ridge, the summit of which is the dividing line between the two protostigmatic areas. One row of stigmata appears on the dorsal or anterior side of the ridge and two on the ventral or posterior. In this case it is very evidently the first protostigmatic area that has failed to divide. I have not been able to find any connection between any of the stigmata of the two posterior rows. Complete division has evidently taken place some time before perforation. In two species of *Polycitor* (*Polycitor*) in the adult blastozoids of which there are many rows of stigmata, two rows appear on each side of the ridge in the larva. All four rows perforate independently of each other. The first stigma of the third row appears a short time before the first of the fourth row, but I have not been able to see any connection between them. This method of origin is shown in the lowest series of stages of the figure.

In the Didemnidæ I have not yet examined larvæ of the necessary age to determine whether all the stigmata perforate independently or whether there is some division of the stigmata, as in *Holozoa*. The stages that I have seen point to an agreement with *Polycitor* (*Polycitor*). In *Amaroucium*, one of the Synoicidæ, two rows appear on each side of the pharyngo-atrial ridge, and I have found no evidence of connection between any of the stigmata. Their origin corresponds with the lowest series in the figure.

The Dictyobranchia and Krikobranchia agree in many points. They differ from the Ptychobranchia in having only one or two primary protostigmata instead of three or more; also in the fact that the first primary divides into two secondary before perforation. The Dictyobranchia differ from the Krikobranchia in that the first primary protostigma divides into four tertiary. The Krikobranchia are peculiar in the great abbreviation of the development. True protostigmata can scarcely be said to occur. They divide into definitive stigmata before perforation.

It might be pointed out that the division of the two secondary protostigmata into four rows in *Holozoa* is not absolutely distinct from the division of the two secondary into four tertiary in the Dictyobranchia. *Perophora* exhibits an intermediate condition. A protostigma corresponds with a row of definitive stigmata. The division of a protostigma corresponds with the doubling of a row of definitive stigmata. Different methods are followed in the two cases, since the condition of the element differs in the two cases.

Summary.

The genesis of the stigmata affords a proper basis for an understanding of the structure of the adult pharynx. A classification based upon the adult structure and development of the pharynx shows marked agreement with the current classification based upon the adult structure of the whole body.

The absolute number of protostigmata in each form is not as good a basis for classification as the number plus the method of formation. Important differences in the method occur. Passing from the Krikobranchia through the Dictyobranchia and Ptychobranchia, a continuous increase in the number of primary protostigmata and a decrease in their ability to subdivide can be seen.

Among the new genera studied may be noticed the following:—

Tethyidæ [*Cynthiidæ*].

Pyura.—The protostigmata develop in the same way as in *Cæsira* [*Molgula*].

Tethyum.—The development is a modification of that in *Cæsira*. The intercalated stigmata arise by abstrictions from the growing points of the ventral ends of the first stigmata.

Boltenia.—An indefinite number of protostigmata are formed, all arising by perforation except the second and fourth, which are intercalated and are formed by the process described for *Tethyum*.

Styelidæ.

Styela.—The protostigmatic condition is the same as for *Boltenia*.

Polycitoridæ.

Polycitor (*Polycitor*).—Four rows of stigmata arise by independent perforation.

Synoicidæ.

Amaroucium.—The process is the same as for the last.

I am indebted to the members of the Biological Board of Canada for providing me with the facilities for collecting material at the Dominion Pacific and Atlantic Stations. Nearly all my material was obtained at those stations.

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On the Origin of the Ascidian Mouth.

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(Communicated by Prof. A. B. Macallum, F.R.S. Received March 3,—Read April 24, 1913.)

A year ago I undertook to determine the origin of the test-bearing and testless parts of the epithelial lining of the siphons of Ascidians. My material consisted of adult *Dendrodoa* (*Styelopsis*), *Cæsira* [*Molgula*], and *Clavelina*, each containing embryos of all stages. These were studied in serial sections.

In the case of the oral siphon, I reached the unexpected result that a large part of the epithelium of the wall of the oral cavity is derived from the primitive neural tube of the embryo. It can readily be understood that the connecting of definite parts of the epithelium of the adult with definite parts of the germ layers of the embryo cannot be done with any great certainty. For several reasons I conclude that the outer limit of the "neural" epithelium of the oral siphon is the margin of the test-bearing epithelium (indicated usually by a distinct ridge or velum), and that the inner limit is the peripharyngeal band or ridge.

The mouth of the Ascidian corresponds strictly with the neuropore of the embryo. The neuropore closes rather early, but re-opens during or after metamorphosis to form the connection between the test-bearing and testless parts of the siphon.

In the three genera investigated I have been unable to find any stage in which the neural tube is separated from the ectoderm at its anterior end, as maintained by Kupffer, Kowalevsky, and Seeliger. I have examined numerous stages (both a year ago and recently), from that of the open medullary tube to the escaped larva, and in all the neural tube is connected with the ectoderm.

The accounts that have been given of the manner in which the stomodæum, the hypophysial canal, and the pharynx become connected are more or less conflicting.

Kupffer (1870) failed to find a neuropore at any stage. He describes the fusion of a stomodæal invagination, with the pharynx to form the mouth.

Kowalevsky (1871) describes the early closure of the neuropore, and derives the mouth from an invagination of the ectoderm, which connects almost simultaneously with the sensory vesicle of the neural tube and with the pharynx.

Willey (1893) describes the early closure of the neuropore and the formation of the mouth by the fusion of the stomodæal invagination with the pharynx. Shortly (in *Clavelina*) or some time (in *Ciona*) after the formation of the mouth, the hypophysial canal connects up with the base of the stomodæum. He does not appear to be perfectly certain of the way in which this latter connection is made.

Seeliger (1904) considers that the neuropore closes early and that the neural tube separates from the ectoderm. Later, the hypophysial canal separates from the sensory vesicle, and grows forward to unite with either the stomodæum, the pharynx, or the junction of the two. But he does not seem to think that the details have been satisfactorily established.

As Willey considered that the point where the hypophysial canal broke through into the stomodæum corresponded with the previously closed neuropore, my conclusions are somewhat in harmony with his. I have not had the opportunity of studying the origin of the mouth in *Ciona* or in a Phallusiid, and possibly in these the neural tube separates from the ectoderm. But in *Clavelina*, which was studied by Willey and Seeliger, I do not believe that the separation occurs. Willey did not consider this point. Seeliger stated that separation occurred, but I am inclined to think that he did not actually investigate it in *Clavelina*. He states, however, that he examined larvæ in which the opening of the hypophysial canal was present, but in which the mouth was not yet broken through.

I have been unable to find a stage in which the canal is connected with the pharynx and not with the exterior. If an actual lumen be implied, Seeliger's statements are confirmed by my findings. But the essential thing is the arrangement of the cells to form a tube, whether closed or open.

When observations are made on entire embryos, the connection of the neural tube with the ectoderm is easily overlooked. Also the fixation of the material or the subsequent handling might break the connection. My material was fixed with Zenker's fluid, and the embryos remained during preservation, embedding and cutting *in situ* inside the adult. This treatment prevented any separation of the parts of the embryo, but made the examination of the sections somewhat difficult.

In the early stages of development the neural tube is widely open in front, but at the time when the tail grows out, the neural tube is closing. The margins of the neuropore come together and from the outside little if any indication remains of the position of the neuropore. In *Clavelina* there is usually a slight depression, more or less filled by testa cells. A section of this stage (fig. 1A) shows the neural tube still connected with the ectoderm, but a definite lumen cannot be traced to the surface of the ectoderm. The position of the original lumen is always indicated by the way in which the cells are arranged. The anterior end of the tube is bent upward to a slight extent (when the neuropore is open the anterior end is straight).

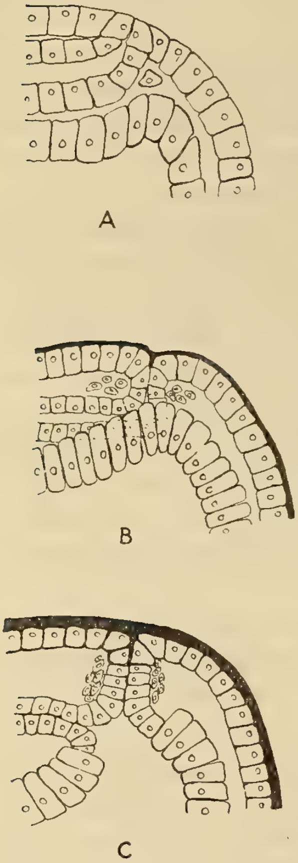

Fig. 1.—Diagrammatic sagittal sections through antero-dorsal part of Ascidian embryos of three different ages, showing transformation of neural tube into wall of oral siphon.

When the anterior end of the neural tube separates into a dilated sensory vesicle (on the right side) and a narrow hypophysial canal (on the left side), it is the latter that retains a connection with the ectoderm. In this stage the anterior part of the canal is bent into the form of a right angle (fig. 1B) and the ventral part of the angle has sunk into the endoderm. The endodermal cells are large and columnar. They separate to make room for the canal. Usually a distinct lumen can be seen in the horizontal part of the canal but not in the vertical part. Grouped around the latter are mesodermal cells forming a characteristic ring. The ectoderm at the neuropore has commenced to invaginate and as the test is appearing the invagination is easily distinguished.

In the next stage (fig. 1C) the hypophysial canal has opened into the pharynx at the angle. The margins of the opening in the wall of the canal have become connected with the margins of the opening made in the endoderm by the sinking of the canal. This causes a great increase in the lumen of the canal at the angle, where this connection occurs. The vertical limb (which we may now call the oral siphon) is still without a lumen and the horizontal limb has little or no lumen. The vertical limb or oral siphon has become longer, partly from a lengthening of the neural part and partly from a further invagination of ectoderm. The layer of test has increased in thickness.

The fully-developed larva shows only unimportant advances on this condition. The oro-pharyngeal opening becomes larger, the oral siphon longer and the layer of test thicker. The oral siphon remains closed till after the metamorphosis.

The above description and the figures are based upon the condition in *Dendrodoa*, but the process is essentially the same in *Clavelina* and *Cæsira*.

In the latter form the extent of the neural tube material is more easily seen than in either of the other forms, since the cells of the neural tube contain much less yolk than those of the ectoderm and endoderm.

There is great difficulty in tracing the further fate of the neural, ectodermal and endodermal cells respectively of the oral region. I believe, however, that the following conclusions are warranted.

At the time when the ectodermal cells begin to invaginate at the position of the old neuropore, test has begun to form on the surface of the ectoderm. The cells invaginated have test on what are or will be their free surfaces. Doubtless they continue the formation of test. If so, the ectodermal part of the adult will consist of the test-bearing epithelium of the siphon, the so-called "reflected tunic." The hypophysial canal of the late embryo and larva opens near the margin of the endoderm (fig. 1C). If this position is retained, the junction of the neural and endodermal regions of the adult will be immediately behind the dorsal tubercle. In this position we have the peripharyngeal ciliated bands. These connect ventrally with the right and left lips of the endostyle. The peripharyngeal bands have every appearance of being a dorsal continuation of the endostyle, split into right and left halves by the sinking down of the neural tube. The subsequent fusion of the latter with the margins of the opening thus formed in the pharynx causes the two halves of the split endostyle to form a circular band at the anterior extremity of the pharynx.

The parts derived from the three embryonic layers will then be,

Ectodermal......	Test-bearing epithelium or "reflected tunic."
Neural	Pretentacular zone.
	Tentacular ridge and tentacles.
	Prebranchial zone and dorsal tubercle.
Endodermal ...	Peripharyngeal bands and parts behind.

This is illustrated in fig. 2, a diagrammatic longitudinal section through

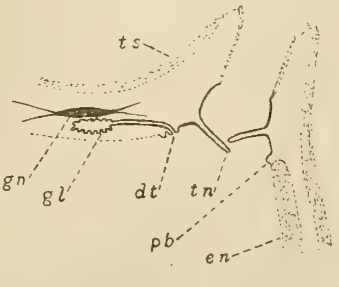

FIG. 2.—Diagrammatic sagittal section through oral region of adult Ascidian. *dt*, dorsal tubercle; *en*, endostyle; *gl*, neural gland; *gn*, ganglion; *pb*, peripharyngeal band; *tn*, tentacle; *ts*, test.

the oral region of an Ascidian, in which all parts, except those of neural origin, are represented by dotted lines.

The three genera that have been the subject of this investigation belong to three different families, and one of them (*Clavelina*) is almost at the opposite extreme of the Ascidian series from the other two. I presume, therefore, to think that this origin of the mouth is characteristic of all or of most Ascidians, and that any exceptions will show only modifications of this. It affords a satisfactory explanation of the opening of the neural gland or hypophysis into the oral siphon.

As to the general significance of this developmental process, I think that comparisons between this process and that to be found in Cephalochordates and in Vertebrates should be used with much caution. I believe that the majority of the homologous organs of the three groups have been evolved independently, and are so remarkably alike because they have been derived from an original similar condition in the groups, this condition being the constitution of the germ plasm rather than the presence of these organs.

With this reservation, I make the following homologies (most of which have been made previously), with the organs in order from behind forwards and downwards.

Tunicate.		Cephalochordate.	Vertebrate.
Ganglion		Ant. end of nerve cord	Main part of brain.
Sensory vesicle	Eye	Eye-spot	Pineal eye and retina of lateral eyes.
	Otolith	...	Auditory vesicles.
Neural gland and duct		Olfactory pit	Hypophysis and nasal cavities.
Neural part of oral siphon		Stomodæum	Stomodæum.

The order is faulty only in the case of the auditory vesicles of Vertebrates. They occur laterally and behind the eyes.

The three groups form a series as to the connection of the different organs with each other and as to the times at which they invaginate from the ectoderm. In the Tunicate they are all connected together and invaginate very early to form the neural tube. In the Cephalochordate the first two and the fourth (the third is absent) are connected. The first two form part of the neural tube, whereas the fourth invaginates later but at the neuropore. The fifth (stomodæum) is separate from the others. In the Vertebrate only the first two are connected together and form part of the neural tube. The others are more or less separate and invaginate later.

These structures are all median and unpaired, except the three sense organs of the Vertebrate, which are usually paired.

These groups also form a series with reference to the amount of rotation of these organs around the anterior end of the body. In the Tunicate they are all dorsal. In the Cephalochordate they are all dorsal except the last. In the Vertebrate the last two are ordinarily ventral and exceptionally nearly all of them may come to the ventral side.

It may be noticed that the condition in the Tunicate strongly supports the view that the neural tube was originally not nervous but a part of the digestive system.

I hope shortly to publish a more extended account of the later embryonic development of the Ascidian. I wish to express here my appreciation of the splendid facilities for research and for the collection of material which were placed at my disposal by the Biological Board of Canada, both at the Atlantic Station, St. Andrews, New Brunswick, and at the Pacific Station, Departure Bay, British Columbia. My material was obtained at these stations.

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Some Investigations on the Phenomena of "Clot" Formations.
Part I.—*On the Clotting of Milk.*

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(Communicated by Prof. E. H. Starling, F.R.S. Received March 6,—Read April 10, 1913.)

(From the Research Institute of the Cancer Hospital.)

Introduction.

During the course of some researches on the bile acids, the observation was made that sodium cholate in 1-per-cent. solution reacts with calcium salts in a characteristic manner. On first addition of the salt solution, the mixture remains clear; on gentle warming, however, it solidifies to form an irreversible gel, the rate of formation of which, other factors being the same, is dependent on the temperature. A series of experiments was carried out to ascertain what influence the concentrations of the various calcium salts exerted on the time required for "clot" formation. These experiments were carried out at a temperature of 50° C. It was found as a result, that the calcium salts, as regards their behaviour to sodium cholate, could be divided into two classes. In the first class are included those salts in the presence of which the clotting time diminishes as the concentration of their solutions is progressively increased. In the second class of salts, the clotting time also diminishes as the concentration is increased, but only up to a certain optimal point; further concentration beyond this point increases the time of clot formation, or even inhibits it entirely. The salts of the first class can be distinguished from those of the second, in that the former increase the surface tension of water (measured against air), whereas the latter cause a diminution of this constant. The greater the diminution of the surface tension caused by salts of the second class, the smaller the range of concentration of the salt solution within which complete clot formation is possible. The actual chemical nature of the clot has not yet been ascertained. It is possibly either calcium cholate or the free acid (formed by hydrolysis of the calcium salt); in either case it is obviously a heavily hydrated aggregate, the formation of which is inhibited by the presence of readily adsorbed substances; the more easily such substances lower the surface tension of water, the more readily are they adsorbed from aqueous solution, and the greater their inhibitory action on the formation of aggregates by the larger molecules contained in the system.*

It is proposed to deal with the subject of the cholate clot in a subsequent publication, but a preliminary account of certain experiments is given in this place, as the results obtained have given rise to suggestions as to the mechanism of a phenomenon of far greater general interest, viz., the clotting of milk by rennet.

From the fact that an aggregation, commonly known as a "clot," can be inhibited by the presence of simple adsorbable substances in a system, the conception arose that in milk the substances necessary for the formation of the clot already pre-exist, but owing to their adsorption of simpler molecules from the system, they do not aggregate, but remain in a state of dispersion. It was thought that a ferment, for which the disperse phase acts as a substrate, could clear the surface of the colloidal particles of adsorbed substances, and thus allow their aggregation to take place; in other words, the ferment could act the part of a scavenger.

If the view here advanced is correct, it should be possible to produce clot formation from a milk protein by the action of calcium salt alone, in the absence of rennet. Indications of such a possibility are afforded by the experiments published more than 20 years ago by Ringer.†

A more or less similar conception underlies the experiments recently published by Hedin and his pupils. The former has shown that ferments, such as rennet, are adsorbed by charcoal, and in this adsorbed state do not exert their full activity; if to such a combination another readily adsorbable substance, such as saponin, is added, the ferment can be freed from its combination with the charcoal and its activity can be partly or wholly restored.‡

Whatever chemical process is involved in the actual clot formation in milk, it is a fact, which is illustrated by various experiments in this paper, that when aggregation is prevented by adsorbable substances, the inhibitory action of the latter appears to be antagonised by the addition of an appropriate enzyme.

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* For other examples of salt action, see Schryver, "Investigations dealing with the State of Aggregation of Matter, Parts I–III," 'Roy. Soc. Proc.,' 1910, B, vol. 83, pp. 96–123.

† 'Journ. Physiol.,' 1890, vol. 2, p. 464.

‡ Hedin, 'Zeitsch. physiol. Chem.,' 1912, vol. 82, p. 175; and Jahnson-Blohm, *ibid.*, p. 178.

The Lability of Caseinogen.

The first experiments on milk clotting were directed towards a preparation of a standard caseinogen solution, upon which the action of alkalis could be quantitatively studied. For the preliminary investigations, two different samples were employed, viz., a commercial preparation made by the "Rhenania" factory of Aix-la-Chapelle, and a preparation made in the laboratory by a slight modification of Hammarsten's process.

The Solubility of Caseinogen in Alkalis.

It has been long known that caseinogen can dissolve in alkalis to give rise to highly acid solutions of what are presumably acid salts. The degree of solubility in alkaline solutions was employed throughout these researches to characterise the various preparations.

In the determination of this factor, no pretence can be made to the accuracy which can be attained in the estimation of the solubility of crystalline substances, owing to various inherent experimental difficulties. The alkaline solution most generally employed was that of saturated calcium hydroxide diluted with its own volume of freshly boiled water ($\frac{1}{2}$ sat. $Ca(OH)_2$). An excess of the caseinogen preparation (usually 1·5–2 grm.) was mixed with 20–25 c.c. of the lime water solution in a bottle, which was then placed over night (usually about 17 hours) on a rotating axis in a thermostat kept at 21·5° C. At the end of this period a saturated solution of caseinogen in lime water containing the excess of undissolved caseinogen was obtained. This mixture could not be directly filtered in the thermostat, as the pores of the filter soon become clogged. In order to get a filtrate, the mixture had to be submitted to prolonged centrifugalisation. An attempt was made to work in every case under as nearly as possible the same conditions. The mixture was consequently always centrifuged for two hours at a speed of 3500 revolutions per minute. After this treatment, the supernatant fluid could be readily filtered through small folded filters. The defects of this method are obvious. It is impossible to work under absolutely constant conditions, and the liquid becomes slightly warm as the result of the centrifugalisation. As there is evidence that the calcium salt of caseinogen undergoes hydrolysis readily when solutions diluter than half-saturated lime water are employed as a solvent, it can be readily understood that errors can arise from the want of constancy of temperature throughout the whole experiment due to the impossibility, with the laboratory appliances available, of carrying out all investigations at exactly the same temperature. Furthermore, the alkaline solutions employed are very dilute, and a relatively small error in standardising such solutions can cause a relatively large error in the solubility determination. Better results would also probably have been obtained had it been feasible to avoid the use of glass vessels. In spite, however, of all these known inaccuracies, the deviations from the absolutely correct figures are so small compared with the changes produced by submitting caseinogen to various treatments, that the lack of rigidity in the experiments does not materially affect the conclusions drawn from the results; it accounts for the small irregularities to be noticed in the various tables of results.

The nitrogen was estimated in 5 c.c. of the filtrate by Kjeldahl's method. The number of cubic centimetres of N/10 acid required to neutralise the ammonia produced will, throughout this paper, for the sake of brevity, be designated simply the solubility of the preparation.

Differences in the Solubility in Alkalis of Various Preparations.

The earliest experiments indicated that great differences existed between the various preparations employed.

A sample of a Rhenania caseinogen showed a solubility in $\frac{1}{2}$ sat. $Ca(OH)_2$ of 5·1, whereas the solubility of the preparation prepared by the modification of Hammarsten's method mentioned above was 21·2. Sodium hydroxide solutions dissolve approximately the same amount of substance as the equimolar (and not equinormal) calcium hydroxide solutions. In this respect caseinogen behaves like other proteins, such as edestin and globulin.

Attempts were then made to account for the differences in the behaviour of the various preparations. A series of products was made from milk, by the method already described, but instead of purifying the precipitated caseinogen by dissolving it in sodium hydroxide, it was treated with other alkalis (normal solutions), from the solutions in which it was precipitated by acetic acid; the procedure was exactly the same as when sodium hydroxide was employed.

No marked difference was found in the solubility of the various preparations thus obtained.

Nevertheless, the numbers found are of quite a different order to the solubility of the Rhenania preparation (5·1). The effect of the treatment of the Rhenania caseinogen by alkali was next investigated. This was dissolved in normal alkali, reprecipitated, and treated by the general routine process (alcohol, ether, etc.). The preparation thus obtained was now much more soluble in $\frac{1}{2}$ sat. $Ca(OH)_2$, viz., 21·1, *i.e.* it was of the same order as that of the preparations made in the laboratory.

Attempts were next made to ascertain the reason of the great differences in the behaviour of the various samples. The action of water at higher temperatures was first investigated.

A sample of caseinogen, freshly precipitated, was treated with alcohol and ether, and then air dried. (Solubility, 21·5.) It was then warmed with water for half an hour at 70°, which caused it to form at first a pasty mass, which became more granular as the heating was continued. It was then treated with alcohol and ether and air-dried. The solubility in ½ sat. $Ca(OH)_2$ was now 10·9, or only a little more than half that of the original preparation. On redissolving the heated product in sodium hydroxide and reprecipitating, a preparation was obtained with the solubility 25·3. Another sample of the heated product was "purified" by solution in ammonia, and the preparation thus produced had a solubility of 22·3. A third sample was "purified" by dissolving in calcium hydroxide. The caseinogen was only very partially precipitated by acetic acid from this solution, and the filtration of the precipitate through paper could only be effected with difficulty. The solubility of this preparation was 12·6.

It is therefore obvious from these experiments that caseinogen, on treatment with hot water, is converted into a product which is considerably less soluble than the original substance in lime water, but which, on "purification" by solution in caustic alkalis and reprecipitation by acids, is reconverted into the substance from which it was formed.

The solubility of heated and unheated preparations in various strengths of alkaline solutions was next determined, and a comparison of the solution capacities of calcium and sodium hydroxide was made.

Saturated $Ca(OH)_2$ solution requires for neutralisation 4·3 c.c. N/10 acid.

An equimolar Na(OH) solution requires for neutralisation $\frac{4·3}{2}$ c.c. N/10 acid.

In the following table the figures indicate the number of cubic centimetres of N/10 acid necessary to neutralise the ammonia produced by the Kjeldahlisation of 5 c.c. of the solution. (For method of determining the solubilities and sources of error, see pp. 462–3.)

Original preparation.		Heated preparation.	
Solubility in $Ca(OH)_2$ solutions.	Solubility in NaOH solutions equimolar with	Solubility in $Ca(OH)_2$ solutions.	Solubility in NaOH solutions equimolar with
Saturated, 46·5	Saturated $Ca(OH)_2$, 41·4	Saturated, 23·9	Saturated $Ca(OH)_2$, 36·6
½ saturated, 22·8	½ saturated $Ca(OH)_2$, 20·3	½ saturated, 11·9	½ saturated $Ca(OH)_2$, 16·4
¼ saturated, 6·6	¼ saturated $Ca(OH)_2$, 10·0	¼ saturated, 4·3	¼ saturated $Ca(OH)_2$, 7·6

It is necessary to call attention to two facts which are indicated in the above table, viz.:—

(1) Whereas saturated lime water dissolves only a very little more than twice as much of the preparations dissolved by the half-saturated solution, the latter dissolves very appreciably more than twice the amount dissolved by lime water of quarter saturation. A similar result has been obtained several times, and it indicates that when the strength of solution is below that equivalent to half saturation, hydrolytic dissociation of the calcium salt takes place. A similar phenomenon is not noticed in the case of the sodium salt.

(2) The solution capacity of sodium hydroxide for the heated preparation is considerably larger than that of the equimolar solution, more especially in the highest concentration. This is in accordance with the fact that caustic alkalis reconvert the product obtained by heat change into its original form.

In order to produce the changes in caseinogen discussed above water is necessary. A preparation of caseinogen shows no change in its solubility in calcium hydroxide after boiling for half an hour with alcohol.

A Systematic Examination of the Action of Water on Caseinogen.

For the purpose of a more detailed investigation on the action of water, samples of caseinogen, each of 5 grm. weight, which had been "purified" twice by solution in sodium hydroxide and reprecipitation with acid (but without any special precautions as to temperature or time of contact with reagents) were warmed at different temperatures (in thermostat) and for different periods with 10 times the weight of water. At the end of the treatment the water was poured off, and the samples were then treated with alcohol and ether, and air-dried. On treatment with alcohol those which had been submitted to higher temperatures, and which on first heating became pasty, were converted into a hard granular mass, and were finely ground with alcohol in a mortar. In the following table the solubilities in ½ sat. $Ca(OH)_2$ of the different preparations are given:—

Solubility of Original Preparation, 22.

Temperatures	37°.	50°.	75°.	100°.
Time of heating—				
½ hour	18·8	16·3	14·4	13·0
1 "	17·5	15·7	11·0	10·8
2 hours	15·8	13·3	10·9	—
5 "	13·7	12·5	10·7	7·4
10 "	11·4	11·5	10·8	6·9
26½ "	11·6	10·1	6·8	4·2

From the above table it will be observed that caseinogen is changed by simply heating with water at incubator temperature. The change proceeds until a solubility denoted by the number 11 is attained. When this point is attained further change appears to be very slow. At higher temperatures, however, further changes are produced, and the caseinogen is converted into a product which is no longer soluble in excess of sodium hydroxide. The products obtained by the action of water at higher temperatures have not been further investigated.

In view of the fact that excess of casinogen preparation was always used in the solubility estimations, and that consequently the saturated solutions of caseinogenate were kept for some time in agitation with this excess, experiments were carried out to ascertain how far adsorption phenomena influenced the final equilibrium. For this purpose, 20 c.c. of saturated solutions of the original preparation and a preparation which had been heated for three-quarters of an hour with water at 75°, in ½ sat. $Ca(OH)_2$, were rotated with 1½ grm. of the various heated products for 17 hours. The mixtures were then centrifuged and the nitrogen was estimated in 5 c.c. of the filtered supernatant fluid. It was found that a small amount of the caseinogenate was thus removed from solution. Thus in the case of the unheated caseinogenate 5 c.c. required, after Kjeldahlisation, about 20 c.c. N/10 acid to neutralise the ammonia produced. Of the same preparation, after rotating with various samples of heated products, 5 c.c. required 17·4–18·2 c.c. In the case of the heated product, 5 c.c. of the caseinogenate were equivalent to 10·0 c.c. of acid, and after rotation with the heated products, to 8–9 c.c. of acid. There was no marked difference in the adsorption capacities of the different heated products. Adsorption phenomena have therefore but little influence on the comparative values of the numbers given in the above table.

The Preparation of "Natural" Caseinogen.

As the above series of experiments have demonstrated the great lability of caseinogen, which, under the influence of water, is readily converted into one or more "metacaseinogens," experiments were next directed to ascertain whether the natural caseinogen undergoes change during the course of its preparation by Hammarsten's method (or modifications of the same).

Efforts were directed towards obtaining a product which should remain for as short a time as possible in contact with the various reagents employed.

To accomplish this end a caseinogen was prepared in the following manner:—Four litres of skimmed milk were diluted with 16 litres of water, and to this mixture 20 c.c. of glacial acetic acid dissolved in 400 c.c. of water were added with brisk agitation. After not more than two or three minutes, when the caseinogen had settled at the bottom of the precipitating vessels, the supernatant liquid was syphoned off, this process being accomplished in as short a time as possible. The precipitate was then washed twice by decantation with several times its volume of ice-cold water. It was then treated with graded strengths of alcohol, up to absolute alcohol, then with ether, and finally air-dried. A very fine light power was thus obtained, which was considerably more soluble in $\frac{1}{2}$ sat. $Ca(OH)_2$ than any of the preparations obtained by the ordinary Hammarsten method, when the precautions described above were not observed.

Whereas the solubility of the Hammarsten preparations in lime water varied as a rule (and the reason of these variations is now obvious) between 20 and 26, that of the new preparation was about 35. The solubilities of a large number of preparations obtained by the new method were determined; the majority of them showed a solubility in $\frac{1}{2}$ sat. $Ca(OH)_2$ which varied but slightly from that given.

Furthermore, the solutions thus obtained both in $\frac{1}{2}$ sat. $Ca(OH)_2$ and the equimolar sodium hydroxide solution, especially that in lime water, had an opaque milky appearance which retained the opacity even after dilution with several volumes of water, which is in marked contrast to the opalescent solutions obtained with the ordinary commercial preparations.

The Lability of the "Natural" Caseinogen.

The factors influencing the changes in the "natural" caseinogen were next investigated.

Effect of Acids.

Ten-gramme samples of the "natural" caseinogen were allowed to stand with 100 c.c. of a 0·1-per-cent. acetic acid solution, which is about the strength of the acid in which the original precipitate is produced. The samples were allowed to stand for varying times with the acid, then filtered, washed with water, and repeatedly with alcohol, to remove the last traces of acid. They were finally washed with ether and air-dried. The following numbers indicate the solubilities in $\frac{1}{2}$ sat. $Ca(OH)_2$ after varying periods of contact with 1/1000 acetic acid:—

Original preparation	35·0
After 1 hour with acid............	27·3
„ 2 hours „	19·3
„ 6 „ „	15·5
„ 24 „ „	11·4

The "natural" caseinogen is therefore very unstable in the presence of acids.

This change in the caseinogen is not due to a ferment carried down with it when it is precipitated from milk, as a product of high solubility in alkali is also obtained when the milk is boiled before the precipitation of the caseinogen. Some preliminary experiments, which need not be given in detail here, indicate that substances present in the milk serum protect the caseinogen from change, when treated with water.

Action of Water on "Natural" Caseinogen.

The action of water on "natural" caseinogen at various temperatures was also investigated in some detail. The method of experiment was exactly the same as that employed in the researches on the action of water on caseinogen prepared by Hammarsten's method (see p. 465). The result of the determinations of the solubility in ½ sat. $Ca(OH)_2$ of the various products are indicated in the following table :—

Solubility of Original Preparation, 35.

Temperatures	37°.	75°.	100°.
Time of heating—			
½ hour	16·8	17·6	14·5
1 ,,	17·2	18·5	13·4
2 hours	14·1	13·8	11·2
5 ,,	14·7	11·7	9·0
10 ,,	13·4	11·8	6·0
26½ ,,	11·5	10·8	4·9

The changes at 51° did not differ materially from those at 37°. It will be observed that up to 75° the temperature has but little effect on the rate of change. Within the first half-hour, however, the caseinogen, even at 37°, breaks down into a product with only about half the solubility of the original preparation in ½ sat. $Ca(OH)_2$. At ordinary room temperature the change is very slow, a preparation left in contact with water for 24 hours having its solubility reduced only to about 30. It is not at present possible to formulate the chemical changes which have been described above. It seems, however, feasible to assume that they are produced by the scission or addition of the elements of water. It is advisable to recall the fact that water is necessary to produce these changes, and the caseinogen itself remains unaltered on boiling with alcohol. If the above conception is correct the presumably more complex and more soluble caseinogen is a poly-acid which first undergoes hydrolysis into a simpler acid, from which, on heating, the elements of water are removed from contiguous hydroxyl groups. On treatment with caustic alkalis, and acidification of the alkaline solution, these elements are added again to the molecule, which is thereby enabled to again form the poly-acid. The relationship, if this view is correct, is similar to that existing between a pyrophosphate and a metaphosphate, a conception which is not improbable when it is remembered that caseinogen is a derivative of phosphoric acid.

Physically there is a marked difference between the saturated solutions (*i.e.* as regards caseinogen) of the calcium salts (containing the same amount of calcium) of caseinogen and metacaseinogen. The former are white opaque solutions which retain their opacity even after considerable dilution, whereas the latter (produced by the prolonged action of water at 37° on natural caseinogen) are translucent, although opalescent.

It may be recalled here that these two forms are readily convertible, one into the other. As might be expected, the nitrogen–phosphorus ratios remain constant. The analyses of the various products are not quoted in this paper. They are also neutralised by the same amount of sodium hydroxide.

The Action of Calcium Salts on Solutions of "Natural" Caseinogenates.

Throughout all the following experiments a saturated solution of "natural" caseinogen in $\frac{1}{2}$ sat. $Ca(OH)_2$ (10 c.c. = 2·15 c.c. N/10 acid) and equimolar carbonate-free sodium hydroxide (20 c.c. = 2·15 c.c. N/10 acid) have been employed. The solutions were always prepared by gentle rotation for 17 hours in a thermostat of 4 grm. of caseinogen with 40 c.c. of the alkaline solution. The mixture was afterwards centrifuged for two hours at a speed of 3500 revolutions per minute, and the supernatant fluid decanted off.

Action of Calcium Chloride Solutions on Sodium Caseinogenate Solutions.—When the caseinogenate solutions are treated with calcium chloride, a precipitate is formed, but only within certain limits of the concentration of the calcium salt. The reactions form therefore an "irregular series," a phenomenon which is not uncommon when a reacting substance is a complex colloid.*

In the following experiment 10 c.c. of the sodium caseinogenate solution were diluted with 10 c.c. of calcium chloride solutions of varying strengths. After standing, the mixtures were filtered through folded filter-papers, and the nitrogen was estimated in 10 c.c. of the filtrate. The results are given in the following table:—

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* These reactions are discussed in some detail in a footnote to a former paper ('Roy. Soc. Proc.,' 1910, B, vol. 83, pp. 97 and 98).

	N in filtrate.	Percentage precipitated.
10 c.c. caseinogenate solution—		
+ 10 c.c. water	28·0	0
+ 10 c.c. N/50 CaCl$_2$	27·4	2·2
+ 10 c.c. N/25 ,,	1·2	95·7
+ 10 c.c. N/20 ,,	1·0	96·4
+ 10 c.c. N/15 ,,	1·2	95·7
+ 10 c.c. N/10 ,,	1·8	93·6
+ 10 c.c. 3N/20 ,,	3·0	89·3
+ 10 c.c. N/4 ,,	28·0	0
+ 10 c.c. N/2 ,,	28·0	0

It will be seen from the above table that nearly complete precipitation takes place only when the concentration of the calcium salt in the mixture lies between N/50 and 3N/20. When the concentration reaches N/4 no precipitation takes place. If, however, a drop of rennet extract is added to the mixture containing the higher concentrations of the calcium salt, precipitation takes place after the interval of a few minutes. Without such addition, no precipitation occurs even after prolonged standing.

The product obtained by the above described reaction appears to be a calcium salt produced by double decomposition between the sodium caseinogenate and calcium chloride. For analysis, the precipitate was washed with 50-per-cent. alcohol, till free from chloride, and then with alcohol and ether, and air-dried. It is a true precipitate which lacks the characteristic physical properties of a clot, which are described below.

Other Methods of Inhibiting Precipitation.—The formation of the precipitate can also be inhibited when the optimal proportions of calcium salt are present by the addition of other substances. In view of the conceptions advanced in the introduction to this paper, it was of importance to investigate more especially the inhibitory action of the substances contained in the milk serum. This was prepared by the addition of sufficient rennet to skimmed milk. As soon as the formation of the clot was complete it was broken up, and the serum was filtered off through muslin, boiled, and again filtered from the protein coagulum. In addition to the action of milk serum, the inhibitory action of Witte's peptone and of glycine were investigated.

Some of the results are recorded in the following table:—

1 c.c. caseinogenate solution—

+0·1 c.c. N $CaCl_2$ solution	+0·9 c.c. water……………………		Immediate precipitate.
,, ,,	+0·7 ,,	+0·2 c.c. serum	Almost immediate precipitate.
,, ,,	+0·5 ,,	+0·4 ,,	Precipitate nearly complete.
,, ,,	+0·3 ,,	+0·6 ,,	Precipitate incomplete.
,, ,,	+0·1 ,,	+0·8 ,,	No precipitate.*
,, ,,		+0·9 ,,	,, *

In the cases where no precipitate was formed in the cold (indicated by an asterisk, *), it came on warming gently (in the hand), but disappeared again on cooling. A reversible reaction apparently takes place. If, however, rennet is added, the precipitate is rendered permanent. The same phenomena were observed in a further series of similar experiments, in which, in one case, a 5-per-cent. solution of Witte's peptone, and, in another case, 10-per-cent. solution of glycine, were employed instead of milk serum.

A further series of experiments was also carried out, in which only half the concentration of calcium salt was present, and the results are as follows:—

1 c.c. caseinogenate solution—

+0·1 c.c. N/2 $CaCl_2$ solution	+0·9 c.c. water	………………	Immediate precipitate.
,, ,,	+0·7 ,,	+0·2 c.c. serum	Turbidity, precipitate on warming which is rendered permanent by rennet.
,, ,,	+0·5 ,,	+0·4 ,,	,, ,,
,, ,,	+0·3 ,,	+0·6 ,,	Precipitate only on warming + rennet.
,, ,,	+0·1 ,,	+0·8 ,,	No precipitate.
,, ,,		+0·9 ,,	No precipitate even on warming and when rennet is present.

Similar results were obtained in experiments in the presence of Witte's peptone and glycine. It will be seen from the above tables that the conditions necessary for the production of a permanent precipitate are somewhat complex, and depend on the relative quantities of inhibitory substances and calcium salt present. They require investigation in greater detail. Whatever interpretation may be given to the particular action, it will be seen that the ferment can produce an aggregation in a system where it is otherwise inhibited by the presence of simple non-colloidal substances, although, perhaps, the interpretation of its action is not as simple as that suggested in the introduction of this paper. This point will, however, receive attention later, in the discussion of the mechanism of clot formation.

Action of Calcium Chloride on the Solution of "Natural" Calcium Caseinogenate. Production of Clot without Intervention of Rennet.

To portions of 10 c.c. of a saturated solution of caseinogen in $\frac{1}{2}$ sat. $Ca(OH)_2$, in a series of test-tubes, were added 10 c.c. of calcium chloride solutions of the following concentrations: N/25, N/20, N/15, N/10, 3N/20, N/5, N/4, N/2. On allowing these mixtures to stand at room temperature no change was observed. On putting the tubes in an incubator, however, the solutions all clotted when the concentrations of the calcium chloride added did not exceed 3N/20. When the concentration was N/5, an incomplete clot formed, but above this limit the liquid remained turbid, and gave no indications of a clot even after prolonged warming in an incubator. The addition of rennet in these cases produced, however, an aggregation even after a short interval. The clots produced all shrank after standing, leaving, when clotting had been complete, a clear supernatant fluid. They possessed, furthermore, a characteristic physical property, which they share in common with the clot produced directly by the action of rennet on milk, and which distinguishes them from precipitated caseinogen, for whereas the latter, on treatment with alcohol, does not alter in the general appearance, the former yield an indiarubber-like mass, which, on further treatment with alcohol, is converted into hard granular products, which can be pulverised only with some difficulty.*

Clot formation can be inhibited not only by excess of the calcium salt, but also by milk serum and solutions of Witte's peptone and glycine. These experiments were carried out with N/25 $CaCl_2$ in milk serum concentrated to half the original bulk *in vacuo*, in milk serum in its original concentration, in 5-per-cent. Witte's peptone solution, and in 10-per cent. glycine; 5 c.c. of these various solutions were added to 5 c.c. of the caseinogenate solution, and the mixtures were allowed to stand for one hour at 37°. At the end of the period no trace of clotting had taken place. The addition of one drop of rennet solution caused the clot to form in less than five minutes. The solutions containing the peptone clotted somewhat more slowly, and the clot formed differed somewhat in character from the other clots, for whereas the latter were firm and shrank in the characteristic manner, the former was more liquid and shrank to a heavy oil.

The behaviour of solutions of calcium and sodium caseinogenate towards calcium chloride were in most respects similar, aggregation taking place within only certain definite limits of concentration and being inhibited by the presence of milk serum, Witte's peptone, and glycine. But whereas the sodium caseinogenate formed precipitates immediately in the cold, which precipitates were not altered in appearance by treatment with alcohol, the calcium salt reacted only on slight warming (the temperature of 25° is sufficient to produce clot formation), and yielded not a precipitate but an aggregation with the characteristic physical appearance and properties of an ordinary milk clot. In both cases rennet could produce aggregation which had been inhibited by the presence of simple adsorbable substances.

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* Ringer (*loc. cit.*), by the action of calcium chloride on a solution of calcium "caseinogenate," produced a curdy deposit in the cold. This reaction differs from the one described above. Ringer's "caseinogenate" solution behaves, in fact, as a "metacaseinogenate" solution, the action of calcium chloride on which is described on p. 473.

Clot formation could also be produced by the action of strontium and barium chlorides. The former acted quantitatively, like calcium chloride, but the latter had a greater range of action, producing a clot when the calcium caseinogenate solutions were diluted with equal volumes of barium chloride solutions in concentrations varying from N/50 to N/5. Even when the concentration reached N/4 a nearly complete clot was formed. Sodium chloride, when added in concentrations of N/50 to 5N (nearly saturated solution), produced no action.

Action of Calcium Chloride on Calcium Metacaseinogenate.

A preparation of metacaseinogen was made by warming "natural" caseinogen for two days with water at 37°. The action of calcium chloride solutions on a saturated solution of this preparation in ½ sat. $Ca(OH)_2$ was investigated. Again an "irregular series" of reactions was produced, but no clots. In the optimal concentrations of calcium chloride only a very partial precipitation was produced, but no precipitate formed (only an opaque fluid) in the presence of excess of this reagent. In these cases, the addition of rennet also produced a precipitate. The general results are indicated in the following table, which illustrates the marked contrast between the behaviour of caseinogen and metacaseinogen :—

	N in 10 c.c. of filtrate.	Percentage precipitated.
10 c.c. metacaseinogenate solution—		
+ 10 c.c. N/50 $CaCl_2$	10·4	0
+ 10 c.c. N/25 ,,	5·5	47·1
+ 10 c.c. N/20 ,,	3·5	66·3
+ 10 c.c. N/15 ,,	3·0	71·1
+ 10 c.c. N/10 ,,	3·8	63·4
+ 10 c.c. N/5 ,,	6·9	33·6
+ 10 c.c. N/4 ,,	10·4	0
+ 10 c.c. N/2 ,,	10·4	0

Other Methods of Obtaining Clots. The General Character of Clot Formation.

The experiments already quoted seem at first sight to fully confirm the theory as to the general action of ferments and inhibitory substances indicated in the introduction. They do not, however, indicate the actual chemical process of clot formation, and the whole subject is somewhat complicated by the fact that caseinogen itself is very labile. Further investigations were therefore necessary to ascertain the nature of the chemical processes involved in clot formation, and to determine whether the change of caseinogen was a necessary preliminary, and whether calcium or any other alkaline earth was an essential constituent of the clot-producing system.

During the course of these additional researches, it was found that it was possible to produce a clot by other methods.

It was found that the calcium caseinogenate solution alone, and without the addition of another calcium salt, could produce a clot on the addition of rennet. This clot formation, which can take place at room temperature, was, however, completely inhibited when the caseinogenate solution was diluted with a equal volume of milk serum, *i.e.* when the milk serum constituents were in only half the concentration in which they exist in milk. In the presence of such quantities of inhibitory solutions, the presence of an additional calcium salt is necessary to produce a clot. As caseinogen can be converted into metacaseinogen by water, it is conceivable that a similar reaction would take place more readily in the presence of rennet. In this case calcium caseinogenate should form a mixture of free metacaseinogen and calcium metacaseinogenate and there would then exist in the system more metacaseinogen than is necessary to saturate all the calcium present, for, as has already been shown above, a saturated solution of this substance in $\frac{1}{2}$ sat. $Ca(OH)_2$ contains only about a third of the amount of nitrogen contained in a corresponding solution of caseinogen. As a matter of fact there is evidence, which is given in greater detail below, that the clot formed in the presence of rennet is produced from metacaseinogen. It is conceivable, therefore, that the clots are formed from the free caseinogen or metacaseinogen directly and not from the calcium salts.

This supposition is also confirmed by other facts. By reference to the table given on p. 464 it will be seen that $\frac{1}{4}$ saturated lime water dissolves less than half the amount of caseinogen or metacaseinogen dissolved by half-saturated lime water, whereas completely saturated lime water dissolves only very little more than double the amount dissolved by the $\frac{1}{2}$ sat. $Ca(OH)_2$. Repeated experiments on the solubility of natural caseinogen in $\frac{1}{4}$ sat. $Ca(OH)_2$ gave solubility numbers varying between 8 and 11 instead of the number 17 or 18, which would have been expected, had the solubility been one-half of that in ½ sat. $Ca(OH)_2$. As these figures indicate hydrolytic dissociation of the calcium salt in low dilutions, it is not unreasonable to suppose that the saturated solution of caseinogen in ½ sat. $Ca(OH)_2$ undergoes a similar hydrolysis when the temperature is raised.* Now attention has been called to the fact that calcium chloride does not produce a clot with calcium caseinogenate at room temperature, but only when the mixture is slightly warmed (*e.g.* to 25°). If, however, the calcium caseinogenate is first treated with carbon dioxide gas, which produces by itself no precipitate, the subsequent addition of calcium chloride rapidly produces a clot in the cold. Finally, it is possible to produce a clot from sodium caseinogenate solutions in the absence of calcium salts. Rennet by itself produces no clot from such solutions; if, however, they are treated first with carbon dioxide, the addition of the ferment solution causes clot production after a short interval, even in the cold. There is evidence that the sodium salt does not readily undergo hydrolytic dissociation (see table on p. 464). Carbon dioxide can apparently decompose the sodium salt and set free sufficient free caseinogen to allow the clotting process to take place.

Saturated solutions of metacaseinogen (prepared by the treatment of "natural" caseinogen with water for 24 hours at 37°) in ½ sat. $Ca(OH)_2$ do not clot on the addition of rennet, and yield only a very faint precipitate on addition of the ferment after previous treatment with carbon dioxide. The action of calcium chloride on these solutions has been already described. By means of these reactions, metacaseinogen can be readily distinguished from caseinogen.

Caseinogen, after solution in alkalis and reprecipitation with acetic acid, can yield solutions in ½ sat. $Ca(OH)_2$, which clot on the addition of rennet or calcium chloride. Furthermore, clots can be produced by the same method from metacaseinogen which has been reconverted into caseinogen by treatment with alkalis (with the usual precautions), provided that the former has not been changed too much by very prolonged action of water either at 37° or at higher temperatures. Where such additional changes have been brought about, there is evidence of the partial scission of phosphoric acid from the caseinogen molecule. In no case was it found possible to reconvert a metacaseinogen into a caseinogen with quite as high a solubility in ½ sat. $Ca(OH)_2$ as "natural" caseinogen. Alkali appears, therefore, to exert some slight subsidiary action.

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* *Cf.* Brailsford Robertson.

The Chemical Nature of the Clot.

As already stated, clots, whether produced by calcium chloride alone, or by rennet alone, can be distinguished from metacaseinogen or caseinogen precipitates by the fact that, when moist, they yield an indiarubber-like mass on treatment with alcohol, whereas the unclotted material undergoes no visible change.

A systematic examination was made of the clots prepared by various processes with the object of determining their relationship to caseinogen and metacaseinogen. The rennet clot was formed by adding 1 c.c. of a commercial rennet solution to 50 c.c. of a saturated solution of "natural" caseinogen in $\frac{1}{2}$ sat. $Ca(OH)_2$. The mixture was made in a high cylinder in the cold, and was then placed in an incubator. After about 10 minutes, the whole had set to a solid clot, which was broken up, filtered off from the liquid, washed with ice-cold water, alcohol, and ether, and then air-dried. It was then boiled with absolute alcohol for about 10 minutes to destroy the ferment, and analysed. A portion was then dissolved in weak sodium hydroxide solution and reprecipitated, after filtration through pulp, by acetic acid, rapidly washed with ice-cold water after precipitation, and freed from water in the usual way.

The clot produced by calcium chloride was prepared by mixing the calcium caseinogenate solution with an equal volume of N/25 calcium chloride solution, and incubating the mixture until the clot had formed. This was then filtered off, washed with 50-per-cent. alcohol until the washings were free from chlorine, then with absolute alcohol and ether, and then air-dried. To free it from calcium it was treated in the same way as the rennet clot, *i.e.* redissolved in alkali (NaOH), and reprecipitated with the usual precautions.

It was found that the clots produced by rennet alone and by calcium chloride alone differed in one important particular, for whereas the calcium chloride clot (purified by solution in alkali and reprecipitation) gave with $\frac{1}{2}$ sat. $Ca(OH)_2$ a milky solution of high solubility (more than 30), which clotted on addition both of rennet and calcium chloride, the purified substance from the rennet clot yielded with the lime water of the same concentration only an opalescent solution (solubility 12·5), which gave a precipitate with an equal volume of N/25 calcium chloride solution, and no clot on addition of rennet.

The rennet clot differed therefore both from metacaseinogen and caseinogen, in that, even after re-solution in alkali, it no longer gave rise to a product capable of giving with $\frac{1}{2}$ sat. $Ca(OH)_2$ an opaque highly concentrated solution, from which clots can be formed.

Experiments were next carried out to ascertain whether the treatment with alcohol had caused the difference between the calcium chloride and the rennet clots. For this purpose a calcium chloride clot was obtained in the form of a dry powder, by the method described above, and then boiled for 10 minutes with alcohol, and purified by solution in alkali and reprecipitated. The preparation thus obtained also gave with ½ sat. $Ca(OH)_2$ an opaque solution of high concentration (29·7) which did not clot on addition of rennet, and gave only an incomplete clot on addition of calcium chloride. Alcohol, therefore, alters both the calcium chloride and the rennet clots. The fact may be recalled that caseinogen on heating with alcohol is not altered, but yields a solution in ½ sat. $Ca(OH)_2$, which readily clots both on addition of rennet and calcium chloride. The clot produced by calcium chloride alone, however, on re-solution in alkali, is readily converted into caseinogen, whereas the clot produced by rennet alone, even after redissolving in alkali, yields a product of low solubility in ½ sat. $Ca(OH)_2$ from which no clot could be produced. It was unfortunately necessary to heat the rennet clot with alcohol to destroy the ferment, and as there is evidence that the calcium chloride clot is also altered by this treatment, the question as to whether the rennet clot can be reconverted into a clottable caseinogen remains for the present unsolved.

Numerous other experiments were carried out with the object of preparing a clottable calcium caseinogenate solution from the rennet clot. The ferment in one experiment was destroyed by heating with water, and then after drying with alcohol and ether was redissolved in sodium hydroxide solution and reprecipitated by acid. In another experiment the clot, after washing with ice-cold water, was directly dissolved (whilst still moist) in sodium hydroxide solution, reprecipitated, dried in the usual way, and then heated for a few minutes with boiling alcohol, a treatment which causes no change in caseinogen. In no case was a product of greater solubility than 17 to 20 in ½ sat. $Ca(OH)_2$ obtained, which is only about half that of natural caseinogen. The solutions were in all cases translucent and yielded no clot either with calcium chloride or with rennet. The evidence obtained so far tends to indicate that rennet alters caseinogen in such a way that it is not reconvertible into caseinogen.

The fact that both descriptions of clot are altered by alcohol, whereas caseinogen is not, indicates that the latter undergoes some change during the process of clot formation. The fact, however, that the rennet clot, even after re-solution in alkali, gives a product of low solubility in lime water, whereas the calcium chloride clot gives one of high solubility (even after treatment with hot alcohol), indicates that the caseinogen under the influence of rennet is converted into metacaseinogen, which undergoes some further alteration, whereas in the absence of rennet it can form a clot without undergoing this change. The clot produced by the direct action of rennet on milk, which was prepared in the same way as the rennet clot from pure calcium caseinogenate solution, behaved in the same way as the latter, yielding after purification a product of low solubility in lime water. It contained, as the analyses* indicate, products other than those derived from caseinogen, carried down from the milk. The analyses show furthermore that the nitrogen-phosphorus ratios in caseinogen, metacaseinogen (unless heated for too long a period), and in the clots produced both by calcium salts and rennet are the same. There is no evidence, therefore, of any proteoclastic digestion produced by the rennet. The change of caseinogen into metacaseinogen is not an essential for clot formation, which can, furthermore, be inhibited by the presence of various adsorbable substances. Owing to the lability of caseinogen, especially under the action of rennet, it is not possible to conclude from the experiments on milk-clot formation that the ferment exerts a direct antagonising influence on the substances inhibiting aggregation, although such an action is not by any means improbable. Nor is it possible from the above experiments to directly formulate the chemical changes which take place in clot formation. The evidence points to the fact that it is the free caseinogen which is changed. This substance, as a complex polybasic acid, can conceivably undergo many changes by the simple scission of the elements of water, and although it is not possible to express in the form of an equation the conversion of caseinogen into casein, such a change does not appear to be at all mysterious when considered from a chemical standpoint.

Summary and Conclusions.

1. A preliminary account is given of the action of calcium salts on sodium cholate (cholalate). When solutions of these substances are mixed, a clot is formed on heating. Investigations were carried out with the object of determining the relationships between the clotting time and the amounts and characters of the calcium salts. It was found that, in the case of those salts which raise the surface tension of water, the greater the concentration of the salt the shorter was the time required for clot formation. In the case of salts which lower the surface tension, on the other hand, increase of concentration decreased the clotting time only up to a certain limit of optimal concentration. Above this limit the clotting time was diminished, or the clot formation inhibited entirely. The more a salt

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* These analyses are not published in this communication.

lowers the surface tension of water the narrower the limits of concentration within which clot formation is impossible. The inhibition of intravascular clotting after peptone injection is probably a similar phenomenon.

2. This inhibition of clotting is probably due to the adsorption of simple molecules by the more complex colloidal substances, which are thereby inhibited from aggregation to form a clot. The results suggested that in other cases, such as that of milk, the materials necessary for clot formation pre-exist, but that aggregation is prevented by the adsorption of simpler molecules from the system. The conception was formed that a ferment, for which the colloidal substances could act as a substrate, could clear the surface of such substances of adsorbed bodies and thus allow aggregation (clot) formation to take place. If such an action of ferments takes place it might be possible to explain the function of the intracellular ferments. If they act in the manner suggested, an aggregation equilibrium in the system—colloids (proteins, etc.), simpler adsorbable substances (extractives, etc.), ferment—would be maintained and would be probably necessary for the maintenance of the normal functions of the cell. There would, in this respect, exist a contrast between the "solid" tissues and the fluids of the body.

3. In attempting to apply this hypothesis to explain the clotting of milk, efforts were made to obtain a "natural" caseinogen. It is already known that caseinogen forms with alkalis solutions of very acid salts, and considerable differences were found in the individual preparations with regard to the amount of caseinogen dissolved by alkalis. The solubility in half-saturated lime water was employed as the criterion for differentiating the various preparations. It was found that if caseinogen is prepared in such a way that it is allowed to remain for as short a time as possible with acetic acid used for its precipitation (1 in 1000), a product is obtained which gives an opaque milky fluid containing nearly 8 per cent. of caseinogen. If such a preparation is heated with water at 37°, or allowed to stand with the acetic acid (1 in 1000) at room temperature, it gives rise to a product, the solubility of which in lime water is only about $\frac{1}{3}$ of that of natural caseinogen. This has been designated "metacaseinogen," the solution of which in half-saturated lime water is opalescent and not opaque. Metacaseinogen can be reconverted into caseinogen by solution in sodium hydroxide and precipitation with acetic acid, provided that the precautions are taken that the precipitate does not remain too long in contact with the acid. The solvent capacity of sodium hydroxide approximates to that of an equimolar (not equinormal) solution of calcium hydroxide.

4. The action of calcium chloride solutions on a saturated solution of

caseinogen in sodium hydroxide equimolar with half-saturated lime water was investigated. It was found that a precipitate was formed (apparently by double decomposition) only when the concentration of the calcium salt was within certain definite limits. The reactions form an "irregular series" similar to many others where one of the reacting substances is a complex colloid. If rennet is added to a mixture in which precipitation is inhibited by excess of calcium salt an action takes place, and a precipitate is formed shortly after the addition of the ferment.

5. If the optimal amount of calcium salt is present, precipitate formation can also be inhibited by the presence of milk serum, Witte's peptone, or even glycine. The addition of rennet to such mixtures can cause precipitation, provided that not too much inhibitory substance is present. The amount of calcium salt also influences the reaction, which depends therefore on the relative quantities of various products present in the system. The relative influence of these substances has not yet been investigated in detail. The precipitating power of calcium salts other than the chloride has also been investigated.

6. If solutions of calcium chloride or the salts of another alkaline earth are added to a saturated solution of natural caseinogen in $\frac{1}{2}$ sat. $Ca(OH)_2$ no precipitate is formed at room temperature. If, however, the mixtures are warmed slightly (to 25°), a typical clot is formed within certain limits of concentration of the calcium chloride. This shrinks, and gives on treatment with alcohol an indiarubber-like mass, and behaves, generally, in a manner characteristic of the milk clot obtained by rennet. In optimal concentrations of the calcium salt, clot formation can also be inhibited by the presence of milk serum, Witte's peptone and glycine. The addition of rennet to mixtures containing these inhibitory substances can cause the clot to form directly.

7. There is reason to believe that the clot is formed from free caseinogen or metacaseinogen and not from the calcium salt. The chief are :—

(*a*) The clot after addition of calcium chloride forms only on warming, and there is evidence that the calcium caseinogenate, under these conditions, undergoes hydrolytic dissociation.

(*b*) The clot can, however, form in the cold, if the caseinogenate solution is previously treated with carbon dioxide.

(*c*) The clot can be formed from sodium caseinogenate solutions, in the absence of calcium, if the latter are treated first with carbon dioxide and then with rennet.

8. Calcium (but not sodium) caseinogenate solutions clot on addition of rennet in the absence of calcium chloride. Clot formation under these conditions is, however, inhibited by relatively low concentrations of milk serum.

9. Solutions of calcium metacaseinogenate are, under optimal conditions of concentration, only incompletely precipitated by calcium salts, and do not clot on addition of rennet.

10. There is evidence that the clot formed on addition of rennet alone is formed from metacaseinogen, as it has a low solubility in half saturated lime water, whereas that formed by addition of calcium chloride alone is formed from caseinogen. Rennet appears also to cause some further change, as up to the present all attempts to reconvert the clot into natural caseinogen (by action of alkalis, etc.) have failed. In this respect, the casein differs from metacaseinogen. Clottable caseinogenate solutions can, however, be readily prepared from clots produced by the action of calcium chloride alone.

11. It is not possible to formulate accurately the relationships of caseinogen, metacaseinogen, and the clots to one another. Analyses negative the suggestion of anything of the nature of proteoclastic digestion on addition of the rennet. The products are possibly formed from one another by the scission or addition of the elements of water from or to the acid hydroxyl groups, and possibly the various products bear the same relation to one another as do, *e.g.* the pyro-, ortho-, and metaphosphates.

12. Although many of the facts appear to support the hypothesis as to ferment actions given above (para. 2), the same cannot be said to be definitely proved by the facts elicited in the study of the phenomenon of milk clotting. The process is rendered more difficult of comprehension by the peculiar instability of caseinogen. There is no doubt, however, that in milk the clot formation depends upon the presence of four series of substances in the system, viz., simple inhibitory substances, colloids, ferment and calcium salt, even if their relative actions cannot be formulated in as simple a manner as that suggested.

On the Action of Radium Rays upon the Cells of Jensen's Rat Sarcoma.

By S. Russ, D.Sc.,* and Helen Chambers, M.D.

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[Plates 12 and 13.]

The experiments of B. H. Wedd and one of us† have shown that if freshly excised portions of mouse carcinoma are exposed to X-rays or the β-rays from a few milligrammes of radium for a comparatively brief period (*circa* 1 hour), the irradiated material will not grow on subsequent transplantation. This line of experimental work has here been extended to Jensen's rat sarcoma, the initial material for which was kindly provided by the Imperial Cancer Research Fund. From several points of view this tumour provides excellent material for the investigations in question. Inoculations are successful in practically 100 per cent., for out of 125 inoculations into normal rats, 124 gave growing tumours; the rate of growth is rapid, tumours measuring 2 × 2 cm. frequently being obtained in 15 days after the inoculation of 0·1 c.c. of tumour emulsion. Spontaneous absorption of the tumours, however, is not uncommon; five disappearances have occurred in 53 rats, all of which were under observation for a minimum period of 20 days.

The Inhibitory Effect of Irradiation by β-Rays.

Thin slices of rat sarcoma from a rapidly growing tumour were exposed between sterile sheets of mica to the β-rays from a source of radium, having an intensity of 1·58 mgrm. per square centimetre. Small pieces of the irradiated material were then inoculated into the right axillæ of a number of normal rats, into the left axillæ of which small pieces of non-irradiated tumour were also inoculated.

The amount of tumour tissue which can be irradiated in this way is necessarily small and the inoculations were, therefore, made with a hypodermic needle and stilet, the pieces of tumour tissue being as nearly as possible of the same size.

This procedure was adopted for the same tumour material for three different periods of irradiation, *i.e.* 30 minutes, 1½ hours, and 3 hours, six animals being inoculated for each series. They were examined at frequent intervals, and fig. 1 gives in outline one half the actual sizes of the tumours which formed.

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* Part of this work was done during the tenure of a Beit Memorial Fellowship.

† 'Journ. Path. and Bact.,' 1912, vol. 17.

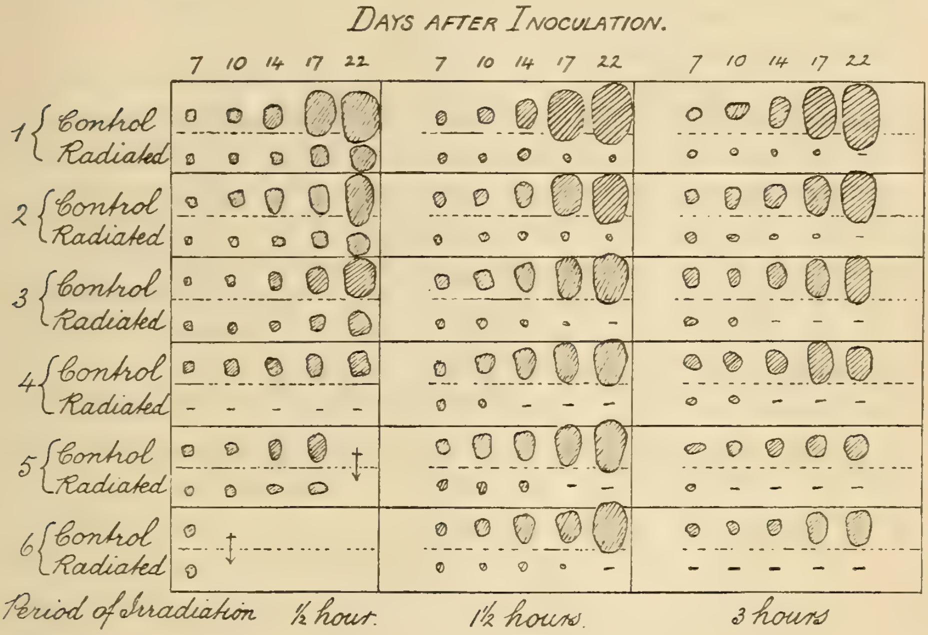


FIG. 1.

It will be seen that as a result of irradiation for 30 minutes there is a slower growth of the irradiated than of the untreated tumour.

When the period of irradiation is increased to 90 minutes, the inoculated material, although apparently increasing in size for some days, was in all cases eventually absorbed in the animal which was simultaneously supporting the growth of the control tumour. Extension of the period of irradiation to three hours ensures the progressive and complete absorption of the tumour cells. There is a close similarity in the action of the β-rays in retarding or preventing the growth of rat sarcoma tissue to the results recorded for mouse carcinoma by Wedd and Russ in the paper to which reference has been made.

The Action of Radium Emanation on Tumour Tissue.

Preliminary experiments were made by mincing a tumour with a Haaland mincer and adding to it sufficient normal saline for the mixture to flow into a small glass bulb. Radium emanation was then supplied in a concentration of about 0·5 millicurie per cubic centimetre. After 32 minutes 0·1 c.c. of the irradiated emulsion was inoculated into each of six rats, 0·1 c.c. of a control portion of the emulsion being inoculated at the same time into the opposite axillæ of the same animals. No tumours developed from the irradiated material, but in each animal a rapidly growing tumour formed from the control emulsion.

An experimental series was undertaken on similar lines in order to determine the dosage of irradiation necessary to prevent the growth of the sarcoma tissue. Tumour emulsion was exposed to 0·275 millicurie per cubic centimetre, samples being withdrawn after 15, 30, and 60 minutes; 0·1 c.c. of each sample was inoculated into each of six rats, 0·1 c.c. of the original emulsion being inoculated into an equal number of other rats, making 24 in all; the average weight of the rats in these four series was 39, 34, 42, and 43 grm. respectively. Measurements of the tumours resulting were made by means of callipers at frequent intervals.

In fig. 2 are recorded the sum of the superficial areas of the growths in the six rats of each series till 27 days subsequent to the inoculations. The tumours in the control animals showed a vigorous growth; in Series I, *i.e.* after irradiation for 15 minutes, a phase of apparent inactivity lasting

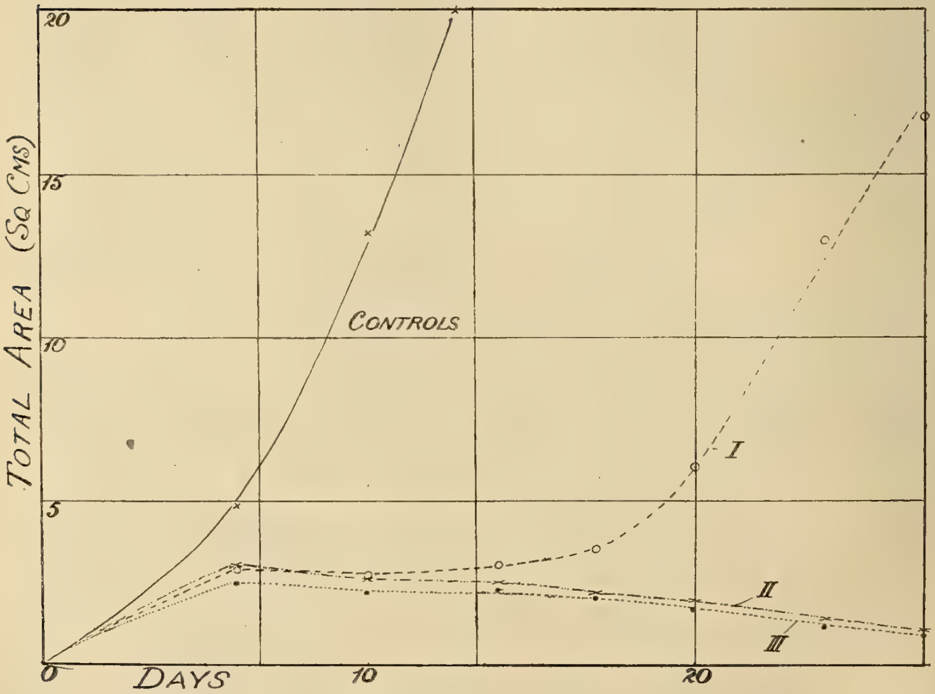


FIG. 2.

about eight days was followed by an almost equally rapid growth. For the more prolonged periods 30 and 60 minutes, *i.e.* Series II and III respectively, the initial reaction, resulting in easily measureable nodules, showed gradual signs of absorption, until after 27 days there was every indication that complete disappearance of the nodules would result. One rat of Series II was killed at this stage, the small nodule was found to consist almost entirely of sarcoma cells (*vide* Plate 12, microphotograph 1).

Continued observations, however, showed that in three of the rats of Series II, nodules remained palpable for a prolonged period and eventually they developed into tumours. In Series III this occurred in one rat and resulted in a fairly rapidly growing tumour. The gradual decline in size of the initial nodules and the subsequent growth of the tumours are depicted for each of these four animals in fig. 3. The full-line curve marked III corresponds to the single occurrence in Series III and the dotted curves to the three rats of Series II. The nodules in the two remaining rats in Series II disappeared after 24 and 81 days. On re-inoculation with 0·1 c.c. of tumour emulsion the first was refractory and the second yielded a growing tumour.

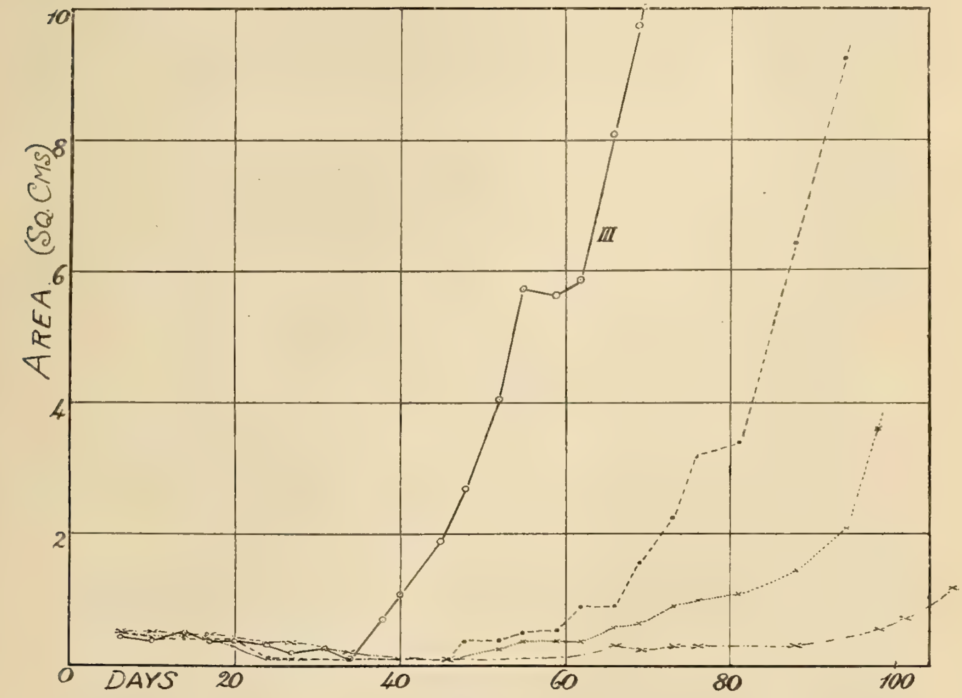


FIG. 3.

Of the five remaining animals in Series III one died, the growths in two of the rats disappeared in 30 and 38 days, the animals proving refractory to subsequent inoculation. The nodules in the two remaining were palpable for 116 days, when they were excised and found to consist of fibrous tissue.

To attempt to interpret the course of events illustrated in figs. 2 and 3 two alternatives may be considered.

If it be supposed that all of the irradiated cells suffer some damage dependent upon their time of exposure, then the irradiated series might be expected to show a general quantitative sequence, and this appears to be the case. On these lines the tumour cells appear to overcome the effect of their irradiation after a prolonged period in the animal body.

On the other hand if it be supposed that some cells are unaffected by the rays, the delay in the apparent onset of growth would be proportional to the time of exposure, and would depend on the number of cells left undamaged.

Although the observations do not allow of a decision between the alternatives, they show that the irradiated cells increase at a slower rate than do the controls. The areas of the tumours were found by actual measurement, and if these areas are raised to the three halves power, numbers are obtained which are proportional to the volumes of the tumours. On the simplest assumption of continuous cell proliferation and reckoning from the time when growth has certainly started, it is found that the mean life period (T) of the tumour cells in the animals of the different series vary in the following manner :—

Control cells (mean of 6 animals) ... . T = 3·4 days.
$\frac{1}{4}$ hour irradiated cells (mean of 6 animals) ... T = 3·9 „
$\frac{1}{2}$ „ „ „ 3 „ ... T = 8·3 „
1 „ „ (one animal) ... ... T = 5·6 „

Attempted Re-activation of Irradiated Tissue.

The changes produced in the tumour tissue by the irradiation may be internal or external to the cells, or the cell boundaries may be affected.

If a tumour be minced by a Haaland mincer, as has been done in this work and the emulsion be vigorously centrifugalised, about 1 c.c. of fluid may generally be pipetted off from 20 grm. of tissue. If the failure of the tumour to grow after irradiation be due to changes occurring external to the cells, such changes might possibly be counteracted by taking tissue that had been irradiated sufficiently long to prevent its growth and adding to it fluid obtained from non-irradiated tumour tissue in the manner indicated.

This re-activation test has been put into operation three times, the extent of irradiation having been 1 hour 4 mins., 2 hours 35 mins., and 5 hours

40 mins., to a concentration of 0·37, 0·40, and 0·37 millicuries per cubic centimetre respectively. The technique followed was practically the same in each case.

To some of the irradiated tumour emulsion an equal volume of fluid obtained from non-irradiated tumour tissue was added and after allowing the fluid to permeate the irradiated tissue for about 1 hour, 0·1 c.c. of the emulsion was inoculated into a number of normal rats (*i.e.* 6 or 8).

To another portion of the irradiated tumour emulsion an equal volume of normal saline was added, and 0·1 c.c. of the mixture inoculated into (6 or 8) other rats to serve as controls. At the same time 0·1 c.c. of fluid only was injected into a number of rats. No reaction was detected when fluid only was injected. In no one of the cases was the attempt at re-activation successful to the extent of the ultimate production of a growing tumour; indicating that the changes occurring in the tumour tissue as a result of irradiation cannot be counteracted by the action of non-irradiated tumour fluid, and that the irradiation probably causes some change in the cells themselves.

Charts of the animals show that 17 days subsequent to the inoculation of the irradiated emulsion treated with fluid, 16 animals out of 22 showed palpable nodules, compared with 4 out of 21 of the control animals. This result suggests that normal tumour fluid has some action upon the irradiated cells, which delays their absorption by the animal although ineffective in re-activating them.

Histological Examination.

To study the histological changes which occur in the irradiated material after inoculation, three series of rats (36 in all) were inoculated on one side with 0·1 c.c. tumour emulsion, and on the other side with 0·1 c.c. of the same emulsion which had been exposed to a concentration of about 0·45 millicurie per cubic centimetre for periods of 20 minutes (*a*), 80 minutes (*b*), and 24 hours (*c*). These times of exposure ensure that the grafts will (*a*) be slightly delayed in growth, (*b*) just fail to develop into tumours, and (*c*) show no signs of proliferation, respectively. An animal from each series was killed each day for the first week, and then at intervals until the 22nd day after inoculation, the control and irradiated tumours were excised and sections prepared.

Microscopical examinations of the emulsions, after irradiation and before their inoculation into the rats, failed to establish any differences between them and the non-irradiated portions.

Control Grafts.

The day after inoculation the tissue of the graft is almost entirely necrotic, only those sarcoma cells at the edge look in good condition. There is extensive invasion of the graft with leucocytes and much inflammatory œdema of the surrounding tissue. On the second day active proliferation of the sarcoma cells at the periphery is evident, and they soon form an encircling ring of growth around the graft. Sarcoma cells extend outwards into the connective tissue and also invade the central necrotic area. By the 6th and 7th day the mass is completely solid and has the structure of the fully developed tumour (*vide* microphotograph 2).

Irradiation for 24 *Hours.*

On the day after inoculation the graft, as in the control, is almost entirely necrotic. The inflammatory changes and œdema set up in the surrounding tissue are less marked; there is also less invasion with leucocytes, and the sarcoma cells are all apparently degenerate. On the third day they can still be detected, but they show no signs of proliferation. On the fourth day the sarcoma cells have completely disappeared. The graft now consists of granular structureless material, a few leucocytes and nuclei alone being left (*vide* microphotograph 3). There is commencing vascularisation and fibrous tissue formation at the periphery.

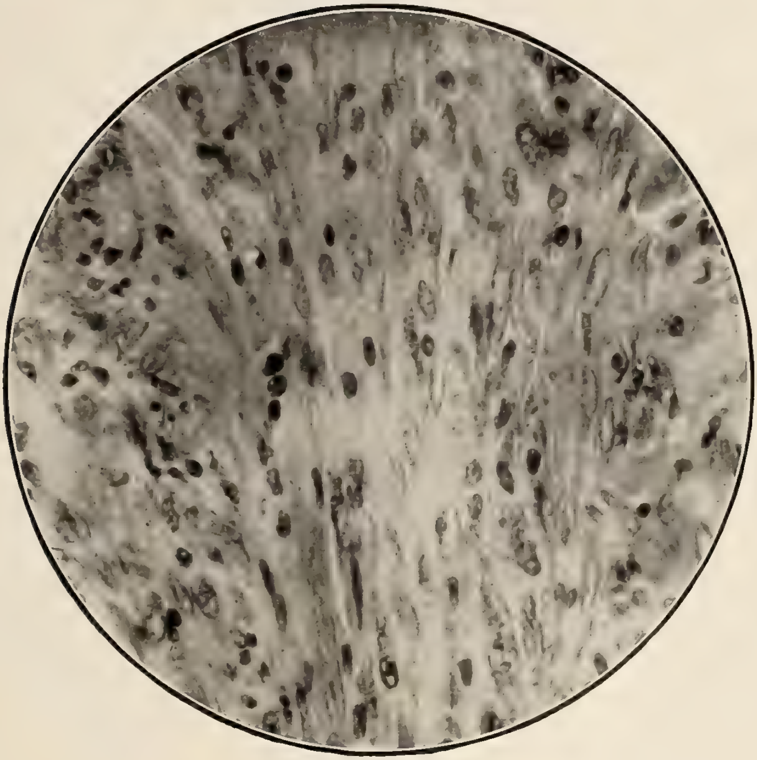
Irradiation for 80 *Minutes.*

On the day after inoculation the reaction of the surrounding tissue to the implanted graft is again less marked than for the controls, and the graft is largely necrotic. Sarcoma cells in good condition can, however, be found at the edge. By the sixth day the graft is almost completely vascularised and many of the sarcoma cells at the edge appear to have proliferated to a slight extent. The condition shows (*vide* microphotograph 4), a distinct contrast with the preceding. At a later stage the graft is largely replaced by fibrous tissue but the sections can be distinguished from those of the 24-hour irradiation series by the presence of a few large sarcoma-like cells embedded in the fibrous tissue.

Irradiation for 20 *Minutes.*

Both the experimental and control grafts formed tumours, the former being the smaller.

The sections of the experimental grafts are similar to the controls throughout this series, except that in the former the rate of proliferation of the cells is delayed. Moreover 11 days after inoculation the irradiated



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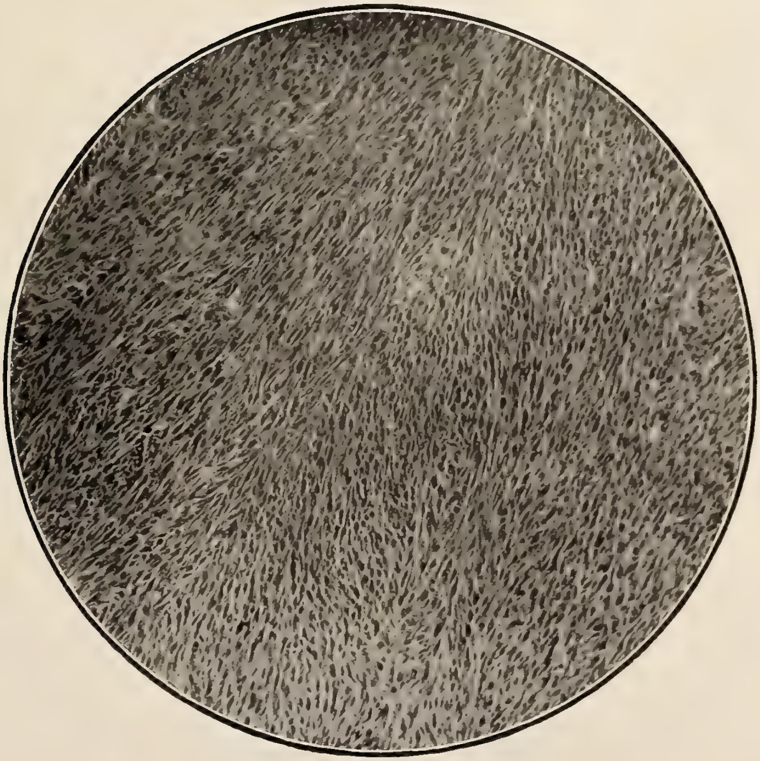

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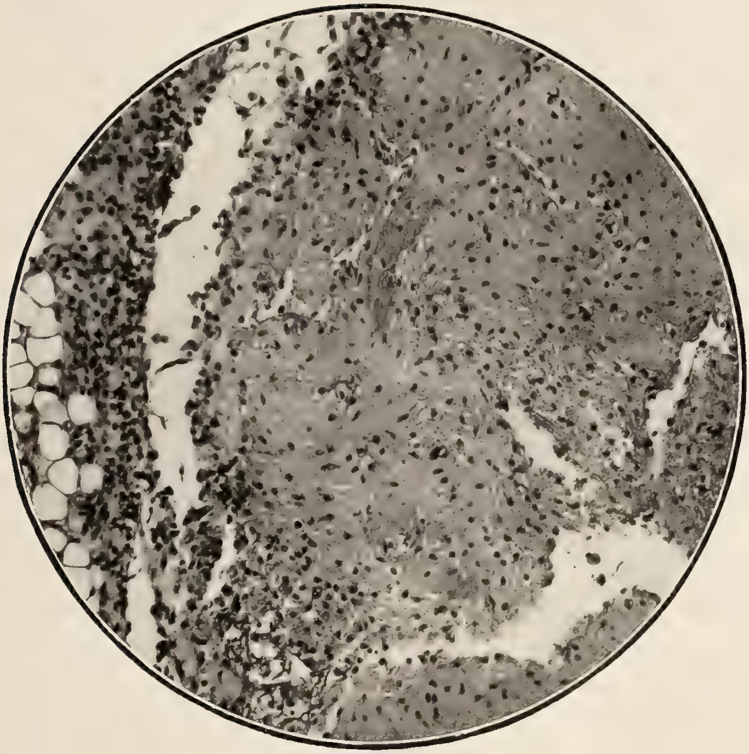

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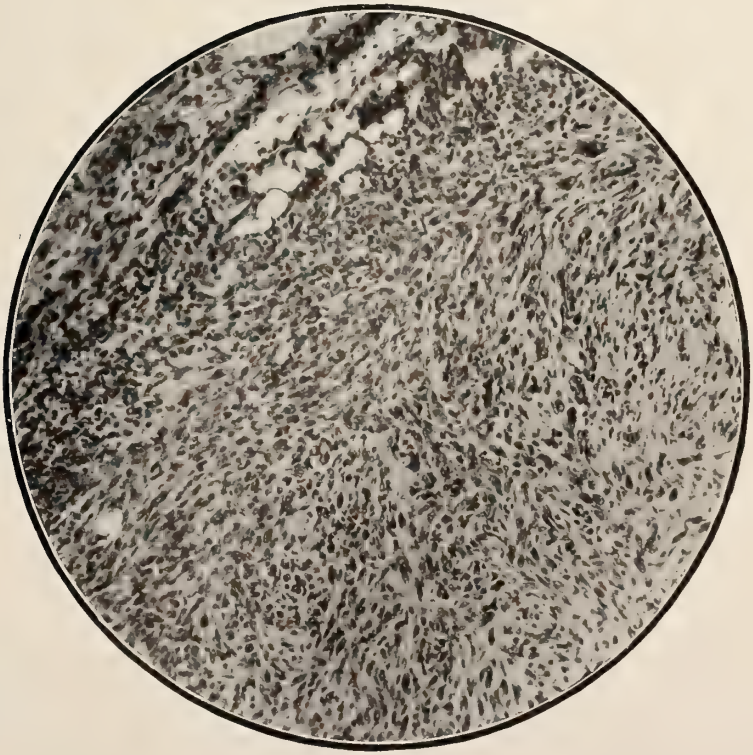

4.

tumour contains numerous very large sarcoma cells, a few of which are multinucleated; they are not found in the actively growing tumours. Cells of this kind have been described by Clunet* and others as occurring in tumours which have been irradiated *in vivo.*

The histological changes indicate that after a long period of irradiation the cells of the growth are killed and are rapidly absorbed. With shorter periods of irradiation, even in cases where no tumour develops, the cells remain at the site of inoculation for a long time, but their capacity for proliferation is diminished. This inability to proliferate is not due, as in immune animals, to failure of the connective tissue to vascularise the graft, but is due to some change in the cells themselves.

Conclusions.

1. Jensen rat sarcoma when exposed *in vitro* to the β-rays from a source of radium of intensity 1·63 mgrm. per square centimetre for 90 minutes, or to radium emanation of concentration 0·53 millicurie per cubic centimetre for 45 minutes, will not grow upon inoculation into normal rats.

2. The sarcoma cells which have been irradiated may remain in the animal body for more than 60 days before giving evidence of growth.

3. Histological evidence shows that failure of the irradiated sarcoma cells to produce a tumour does not necessarily indicate their destruction at the time of inoculation.

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* Clunet, 'Tumeurs Malignes,' 1910.

On Light-Sensations and the Theory of Forced Vibrations.

By George J. Burch, M.A., D.Sc. Oxon, F.R.S.

(Received April 19,—Read June 26, 1913.)

Every hypothesis, whether mechanical, photo-chemical, or ionic, concerning the connection between the light-waves and the sensations they evoke, must of necessity rest ultimately on the theory of forced vibrations. It seemed probable, therefore, that a model illustrating the production of forced vibrations over a range comparable with that of a light-sensation might be of service for teaching purposes, and might prove suggestive in studying the phenomena of vision.

The apparatus now described was made for me in May, 1909, by Mr. H. Davis, the Assistant for Manual Instruction at University College, Reading, and was used in my lectures both in Reading and in Oxford, but no account of it has been published. From a light wooden bar, A (fig. 1), pivoted at the two ends, hang a number of grey silk ribbons, varying in length from 11 to 44 cm. The longest, which represent the red end of the spectrum, have therefore a period twice as long as that of the shortest, which correspond to violet. Each ribbon is weighted at the end with a strip of lead.

The red sensation is represented by a scarlet ribbon occupying the

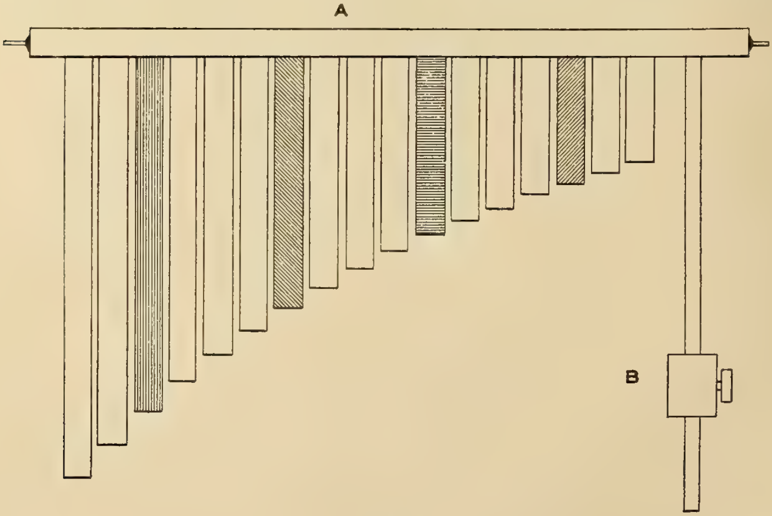

Fig. 1

Side Elevation of Oscillating Bar A, with Silk Ribbons of different lengths acting as Resonators to the Pendulum with Movable Weight B.

position corresponding to Fraunhofer's C-line, the green sensation by a green ribbon near the Fraunhofer *b*-line, and similarly the blue and the violet by a ribbon of each colour in the corresponding region of the spectrum In the actual model there are five grey ribbons between every two coloured ones—in the diagram only three are drawn, for clearness. The bar A is made to oscillate by a heavy pendulum B, with a movable bob fixed by a thumb-screw. If this is tuned to the period of the green ribbon, although those on either side respond more or less, the maximum amplitude is attained by the green, which, according to the theory of Thomas Young, is most strongly excited by light of a certain wave-length.

If the pendulum is tuned for the yellow, then the grey ribbon underneath the yellow portion of the bar indicates by the amplitude of its oscillation the position in the spectrum of the impressed vibration, while the red and green, representing the colour-sensations, swing moderately, being both, as Young said, excited, though to a less degree, by yellow light. It makes the experiment more striking if the bar A is painted with the complete series of spectral colours, and the principal Fraunhofer lines marked upon it in black in their proper positions.

For some reason, perhaps connected with the fact that the ribbons swing so near each other and that eddies are formed by their edges, friction is relatively greater at small than at large amplitudes, so that to some extent the effect of its increase on the range of resonance may be seen as the oscillations subside. For greater differences I use a set of oscillators with lighter weights.

For purposes of demonstration it is necessary to show the effect upon such a system of resonators of light of more than one wave-length. To do this two heavy pendulums, C and D (fig. 2), are connected by light wooden rods, E and F, to the ends of the cross-link G, from the middle of which a third rod H leads to a crank-pin on the bar A. The heavy bob of the pendulum B being removed, the stem of it, which is quite light, serves as an index to show the compound nature of the motion imparted to the bar A, the movements of which often appear strikingly irregular. But each pendulum is responded to by the resonators in tune with it, as though the others were still.

Quite apart from any theories of colour-vision, the apparatus demonstrates in a striking manner the phenomena of forced vibrations. The change of phase according as the natural period is greater or less than that of the impressed force is well shown, and it is particularly instructive to start with a considerable difference of period between the pendulums C and D, and gradually bring them into unison.

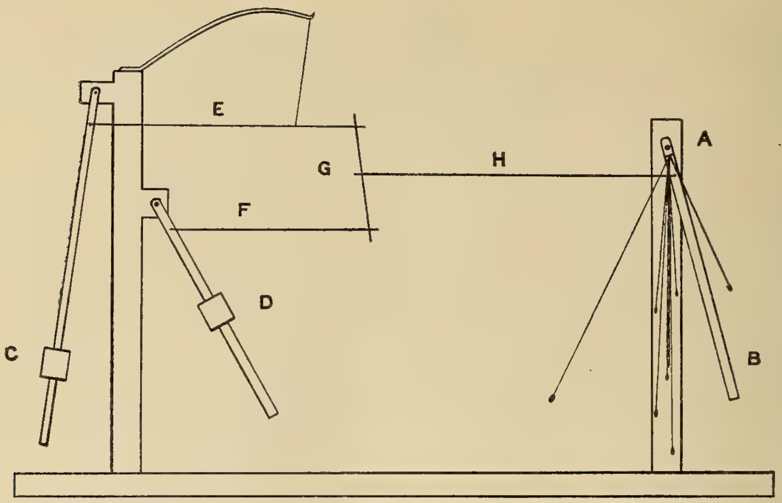

Fig. 2

End Elevation of Oscillating Bar A, showing its connection, by the link-work, E, F, G, H, with the Pendulums C and D, the two Resonators in tune with which are oscillating violently, and the rest scarcely at all.

The periodic variations of amplitude in the response which die out after the impressed force has been in action for a little while are easily seen, and, in fact, the apparatus affords an excellent illustration of §47 and §48 in Volume 1 of Rayleigh 'On Sound.'

For my present purpose the main interest of the apparatus lies in the possibility of utilising it to elucidate one of the most difficult problems of colour-vision—the problem, namely, of white light.

Newton's discovery of the physical fact that the prism separates the beam into rays of different refrangibilities and different colours affords no explanation of the physiological fact that any one of these colours, even the most brilliant, should disappear with absolute completeness in presence of the others. Hering's theory of the antagonism of red and green, and of blue and yellow, is, from this point of view, a most natural one. It would be simple enough, on Young's theory, to explain why all colours tend to white by very strong light, for each of his three hypothetical "nerves" is assumed to be affected, though to a different degree, by light of all wave-lengths, so that we have only to suppose that, with a sufficiently strong stimulus, all three "nerves" are almost equally excited. But this leaves us without any valid explanation of that other fact, that by very feeble light all colours tend to grey.

In some respects the phenomenon of colour is more striking than that of whiteness, and probably, if it had been possible for the spectroscope to be invented before any theories on the subject were thought of, the problem would have rather been to explain why, with a certain intensity of illumination, light of different wave-lengths should appear brilliantly and variously coloured, whereas with less light or with more the colours fade.

The existence of this optimum intensity for the excitation of the sense of colour is very noticeable when calibrating an ordinary students' spectroscope with the Fraunhofer lines by direct sunlight. The act of measuring produces sufficient fatigue to make the spectrum appear pale. If now the instrument is directed towards the sky or a cloud, the colours instantly become rich and brilliant, though it may be necessary to open the slit wider before there is light enough to see them.

I propose to show how this entire range of phenomena—from the scarcely visible band by feeble light to the brilliantly coloured spectrum with optimum intensity, and the washed-out colour with bright sunlight—is in strict accordance with the laws of forced vibrations.

It is, of course, understood that any hypothesis as to the manner in which the ethereal vibrations we think of as light give rise to the sensations we know as light would naturally be expressed, in the first place, in terms of the electromagnetic theory. Inasmuch, however, as the same laws apply to all kinds of harmonic oscillations, whether electrical or mechanical, it will be more convenient to retain the phraseology proper to the mechanical model.

Evidently, the action, whatever it be, must take place through those processes which we commonly regard as chemical—processes, namely, in which some rearrangement of atoms, either within the molecule or from some other molecule, is brought about.

There is a very clear statement by Kühne of the Opto-chemical Hypothesis in Hermann's 'Handbuch der Physiologie,' vol. 3, p. 327. According to him, the opto-chemical hypothesis regards the visual cells as carriers of chemically decomposable materials called visual substances, which, however, have no effect upon the visual cells as long as they are undecomposed. But the hypothesis ascribes to the decomposition products resulting from the action of light on these substances the power of chemically exciting the protoplasm of the visual cells.

This excitation might conceivably result from the act of decomposition by light of the visual substances, but inasmuch as the effects do not instantly cease with the removal of the stimulus, it would appear that the cause is to be sought rather in the material action of the decomposition products.

In applying to this theory the elementary principles of chemical dynamics, we must carefully separate the initial electro-chemical action from the resultant physiological action. The production of the active decomposition products which excite the retina takes place, doubtless, according to formulæ capable of exact and more or less simple numerical expression. The regeneration of the sensitive material, whether due to a natural tendency to revert to its original condition or whether effected, as seems more probable, by processes of metabolism, is still conceivably capable of numerical expression. But between these actions, which may be classed as electro-chemical, and the resultant sensation there is a whole group of modifying causes, which must, for the present, be kept outside our attempts at analysis.

There arises, in this connection, a question of general interest in physiology that has not, so far as I know, been fully dealt with. Is the excitation of a tissue by a chemical compound a process of the same character as a chemical reaction? I do not refer to such crude foreign substances as dehydrate or coagulate, or otherwise damage the tissue, but such as excite quite normally its characteristic functions. There must be some end to the activity of the exciting substances in the eye, else, once separated, they would go on exciting sensation indefinitely. Either they recombine or are washed away or their energy is transformed into that of the sensation. I do not attempt to decide this problem, but have so stated the theory that it would not be affected by it.

I. *Opto-chemical Processes.*

Let n = the number of molecules of the visual substance not yet acted on by light.

x = the number of molecules of the decomposition products capable of exciting the retina.

e = the number of molecules of decomposition products used in producing sensation.

The known data are insufficient for a complete statement of the problem—for instance, it would be necessary to know whether any molecules of x remain ultimately unaccounted for by e—whether they are neutralised, destroyed, or simply washed away by the circulation and dispersed.

For our present purpose it is sufficient to note that each of the variables is subject to conditions of equilibrium governed by the ordinary laws.

Then dn/dt = the rate of supply of the visual substance,
and dx/dt = the rate of demand upon it.
Also de/dt = the *physical* intensity of the excitation.

If dn/dt is greater than dx/dt the store n of molecules capable of being acted on by light increases, and we have, when dx/dt is zero or very small, the condition of dark-adaptation, in which n reaches its maximum.

In the dark-adapted eye we must regard the compound in which the forced vibration is produced, as in a state of maximum concentration, and by all the analogies of electro-chemical action, this must correspond with increased resistance, or, in the mechanical model, with increased friction.

In order therefore to obtain a graphic representation of the meaning of dark-adaptation, we may compare together a series of curves of forced vibrations with different values of the coefficient of friction.

Let it be assumed that the quantity of the exciting substance set free from the sensitive material may be taken as corresponding to the kinetic energy of the forced vibration induced by the action of the light, the expression for which is

$$B = \frac{1}{\Delta^2 + 4k^2}, \quad \text{where} \quad \Delta = \frac{n^2 - p^2}{n} = \left(\frac{n}{p} - \frac{p}{n}\right)p,$$

p being the natural period of the resonator, n that of the impressed force, and k representing friction.*

The ratio p/n corresponds to the musical interval between the natural period of the resonator and that of the forced vibration, and as the expression for Δ is symmetrical with regard to the ratio n/p it follows that if values of B are taken as ordinates and ratios n/p as abscissæ, we shall have for each value of p a curve symmetrical about its apex.

This consideration naturally suggests plotting the spectrum by what may be called the "keyboard" system, in which, as in the piano, equal horizontal distances correspond to equal musical intervals. Except in general illustration of the relation between ultra-violet and infra-red rays I do not think this method has been used. It seems to offer certain advantages in dealing with problems of colour-sensation.

Fig. 3 shows four hypothetical colour-sensation curves plotted on this

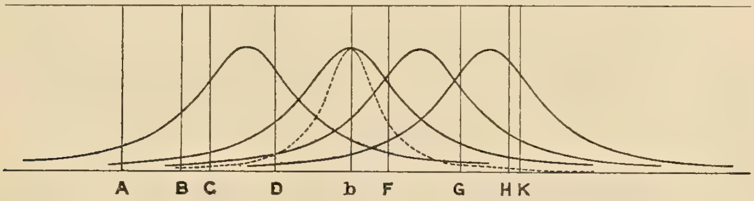

FIG. 3.

Resonance Curves of the Colour Sensations Red, Green, Blue, and Violet, with the coefficient of friction, $k = 0{\cdot}2$. For the dotted curve $k = 0{\cdot}1$, but the ordinates are reduced to one-quarter of their full value by the shunt factor.

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* Barton, 'Handbook on Sound,' p. 145.

system. They represent the four colour-sensations described in my paper on "Artificial Temporary Colour-Blindness."[*]

Inasmuch as according to the theory these curves must be symmetrical, I assumed the apex of the green sensation to lie midway between the two ends of it, and similarly with the blue sensation. Then that part of the yellow where neither red nor green predominates must be where the tangents to the two curves are equal and opposite. In this way I got a position for the apex of the red sensation, and in like manner for the violet sensation by means of the blue. It may serve to fix our ideas if I refer to the illustration of the musical scale. Taking E as the apex of red, green would be approximately at G, blue at A, and violet at B.

It is noteworthy that green, blue, and violet are practically equidistant and much closer together than red and green—red in fact might almost be taken to correspond with E♭.

In order to assign a value to the coefficient of friction I was guided by the keyboard interval over which the forced vibration must extend. After a number of trials I decided to take $4k^2 = 0{\cdot}16$ as a trial value, giving each of the four colour-sensations the same coefficient of friction.

It will be observed that the transition from blue to violet occurs at G, that from green to blue at F, and that from red to green a little above the D line—or exactly at the D line if the centre of the red is placed at E♭. In this case, too, the red would meet the blue about the *b* line, as it does during artificial green-blindness.

Fig. 4 shows the effect of increasing $4k^2$ to $0{\cdot}60$. Each one of the curves is lower, but it extends over a greater range of wave-lengths in proportion to its height. This agrees perfectly with the well-known fact that "by feeble light all colours tend to grey."

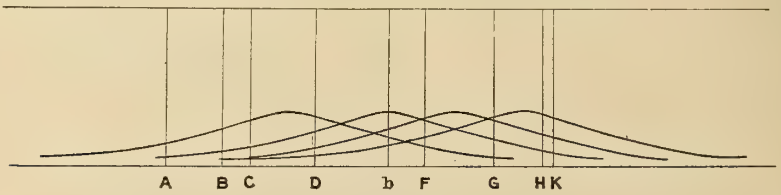

Fig. 4.

Resonance Curves of the Colour Sensations Red, Green, Blue, and Violet, with the coefficient of friction, $k = 0{\cdot}4$ nearly, *i.e.* $4k^2 = 0{\cdot}60$.

In my paper on "Colour-Vision by Very Weak Light"[†] I have noted the changes which occur in such cases between the boundaries of the colour-sensations owing to the fact that they are not all affected in the same proportion, and also the general effect of the increased extent in the spectrum of the several colour-sensations. "The colour must look pale under feeble illumination owing to the presence of three if not of all the constituents of white."

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[*] 'Phil. Trans.,' B, vol. 191, pp. 1–34.

[†] 'Roy. Soc. Proc.,' 1905, B, vol. 76, p. 214.

If this view is correct it would explain a rather puzzling fact that I have noted in my investigation of cases of colour-blindness.* I found it necessary to specify not only those cases in which a colour-sensation was deficient, but those also in which it was of greater extent than usual. The Swiss girl therein referred to had practically but one colour-sensation, because her green so greatly exceeded the normal in spectral extent, yet by suitable fatigue it could be reduced so as to reveal her possession of the other sensations. We have only to suppose that the supply of sensitive material to the green end-organs was so copious as to cause a quite unusual concentration, with correspondingly large coefficient of friction. In such a case the eye would be with respect to green in a condition of dark-adaptation, with this difference, that no ordinary demand could overtake the supply and reduce the store, n, of molecules of visual substance to the normal amount.

In other words $dn/dt - dx/dt$ was positive for all ordinary values of dx/dt.

But it is clear that this might arise either from dn/dt being large, or from dx/dt being abnormally small.

I consider that I have met with cases of both kinds. In one, of which I shall give a full account in my next paper on "Cases of Colour-Blindness," there was monochromatic vision in one eye only. This enabled me to ascertain that the sensation was unmistakeably one of whiteness, with a little colour at the two ends of the spectrum. But the whole intensity of the light-sensation was extremely low although the transparent tissues were perfectly clear.

In the same way we may explain the fact noted in my paper on "Artificial Temporary Colour-Blindness"† that with some people the "overlaps" of the colour-sensations are very large, and with others almost non-existent, and also that with some the overlaps are large between red and green and small at the other end of the spectrum, so that to them yellow is an important colour, and with others there is little or no overlap between red and green, and large overlaps in the green, blue, and violet region. It seems to indicate that the coefficient of friction is constitutionally large in these people either for all the colours or for those to which the large overlaps belong. And by the expression "constitutionally large," I mean large not because any temporary cessation of the demand dx/dt for the exciting molecules has allowed a large store, n, of visual substances to collect, but because the metabolism is so active that the rate of supply dn/dt of the visual substance is greater than in most people. In quite a number the rate of supply of the visual substance for the blue is so great that it is not recognised as a separate sensation, but confused with violet.

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* 'Phil. Trans.,' B, vol. 199, pp. 239 and 250.

† 'Phil. Trans.,' 1899, B, vol. 191, p. 1.

According to this hypothesis, the store n of molecules of the visual substance would be governed by an equation of equilibrium between the causes tending to use, destroy, or disperse the exciting molecules, and those by which they are formed, so that it would have a definite value for every constant intensity of illumination and of physiological condition. In ordinary dark-adaptation we should therefore have a large accumulation n of molecules of the visual substance, so that on coming into a quite moderate light the rate of formation of exciting molecules dx/dt may for a short time be greatly in excess of its ordinary value for that degree of illumination, producing a sensation vividly described by the French word *éblouissement.*[*]

This gradually passes off as the store n falls to its equilibrium value for that rate of demand dx/dt, when the eye reaches its new condition of adaptation, the curves of the colour-sensations changing into those belonging to the coefficient of friction proper to the concentration represented by the new value of n.

As the intensity of the light increases the equilibrium value of n grows less—the resonance becomes more free and the curves grow peaked, so that a condition is reached represented by fig. 5. Each colour is brilliantly distinct and separated from the rest, dominating its own region of the spectrum. This is the optimum intensity for the excitation of the sense of colour. What happens when the light is still brighter will be described in the next section.

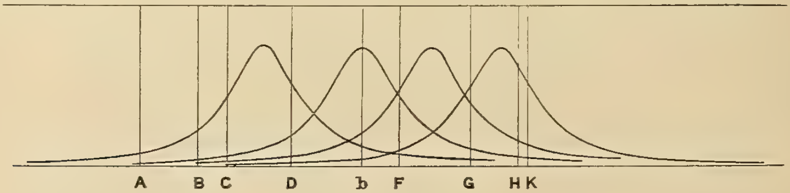

Fig. 5.

Resonance Curves of the Colour Sensations Red, Green, Blue, and Violet, with the coefficient of friction, $k = 0{\cdot}15$.

Before passing on, it must, however, be noted that according to the opto-chemical hypothesis we must imagine the decomposition products to be formed by the action of the light, independently of whether or when they are used.

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[*] See also, for the physiological aspect of this, p. 501.

The effect of a sudden flash must be to set free a definite quantity of exciting molecules which will continue to produce sensation until their power is exhausted, or they are dispersed. This may occupy several minutes even after a momentary flash, and much longer for a more prolonged exposure to light. The phenomenon may be detected as a very brief after-effect even with light of low intensity.

Using the terms already employed :—

dx/dt is conditioned by the intensity of the illumination and the store n of exciting molecules.

de/dt represents the *positive after effect* so soon as dx/dt becomes zero, *i.e. when exciting molecules are no longer produced.*

This agrees with Fechner's theory as described by Helmholtz. And at this point it becomes necessary to take into account also the physiological elements of the visual sensation.

II. *Physiological Elements of the Visual Sensation.*

Thus far, the conditions discussed relate to one process only of the opto-chemical action, namely, the setting free of the active decomposition products which excite the retina from the bland visual substances which have no action upon it.

The other process, necessary to complete the visual effect, is physiological. It is complex, including the excitation of the sensitive elements of the retina by the active decomposition products, the transmission to the central organ of the response, and its translation into conscious sensation. Moreover, there is strong evidence of the existence of a protective mechanism whereby the intensity of the stimulus is regulated.

This physiological process is curiously distinct from the first-described opto-chemical process. The rate at which the exciting substance can be produced far exceeds that at which it can be used up. This can easily be shown by means of a photographic exposing shutter fixed in the window of the dark room. The shutter is set to give an exposure of known length, and the observer looks through it at the sun's disc reflected in the mirror of the heliostat. The positive after-image so produced lasts many times longer than the flash. I have made some attempts to establish a relation between length of flash and duration of after-image, but have had to relinquish the work for the present before getting complete data.

The store of exciting molecules set free by the action of the light is used up at a rate depending apparently on the concentration, so that it is rapid at first and dwindles down to nothing. It follows from this that during continuous illumination the sensation of light at any instant is not due exclusively to the light falling on the eye at that instant, but includes also the remainders left over from previous illumination.

Now in the electrical stimulation of nerve, and nerve-muscle preparations, after a quite moderate strength of stimulus has been reached no further increase causes any modification of the response. This was abundantly evident in the experiments by Gotch and myself with the capillary electrometer—and, moreover, there was no sign of "fatigue" of nerve unless the excitation was excessively great. It is fair to conclude that when, under the action of light, the exciting decomposition products reach a certain concentration, no further increase adds anything to the intensity of the resultant light-sensation.

This may be far stronger than is pleasant, just as the muscular contractions during cramp may be excessively painful, but it does not seem probable that any compound produced as the result of a normal physiological process can be of such character or of such concentration as to destroy the tissues in which it originates. We should thus have a maximum limit to the possible intensity of the light-sensation—a limit independent of the particular physiological condition of the eye at the moment. Were it not for this the theory of forced vibrations would indicate that with intense and long-continued illumination the several colour-sensations must stand out more and more distinctly instead of becoming paler and tending towards white.

But there is evidence of another factor, purely physiological, which I may term the shunt-factor, whereby the strength of the sensation is governed.

I am inclined to think that this factor, manifesting itself under various conditions, affords the explanation of several quite different phenomena.

Thus Charpentier's bands may be taken as evidence that the sudden onset of a fairly bright illumination over a large surface results in a sensation of intermittent intensity. Shelford Bidwell's experiments with pigments and my own with spectral colours show that each colour-sensation acts independently of the others in this respect. Purkinje's recurrent images, especially in the striking form described by McDougall,* exhibit the same thing in connection with the positive after-effect. Some controlling mechanism is set in action whereby the positive after-effect, as it dies away, is periodically shut off like the sound in the swell box of an organ. And this action, like that of the bands of Charpentier, gives fairly rapid alternations. I take it to be a spasmodic excitation of the negative after-effect.

A similar but much slower periodicity may be observed in connection with the fusion of binocular images, especially when both are needed to complete some familiar outline, *e.g.* a cross. The phenomenon occurs not only with the direct image but with its positive after-effect, the waxing and waning of which was known long before the time of Thomas Young.

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* 'Journ. Psychol., 1904, vol. 1, part 1, p. 91.

When making my experiments on artificial temporary colour-blindness I particularly noticed that the positive after-effect of a brilliant monochromatic light over a large retinal area showed nothing of this periodicity but died out steadily and gradually.

Putting together these facts I have suggested in my paper on "Areal Induction,"* that they indicate the existence of a protective arrangement in the retina by which the eye is shielded from the sudden effects of too strong a light. I now submit that there is sufficient evidence to identify this protective action with the negative after-effect of which it is a special case, and that the other phenomena to which I have just referred come under the same category.

The negative after-effect is so generally referred to as synonymous with fatigue that some reference to that aspect of the problem is imperative. The sensitive materials being used up and exhausted, the eye is supposed to be rendered locally less sensitive for a while. But the photo-chemical conditions indicate a state of increased activity, for if the store n is exhausted, dn/dt reaches its maximum, and during continuous steady illumination $dx/dt = dn/dt$.

But it is easy to show that during a bright summer day the quantity of sensitive material used without causing any sensation of visual fatigue greatly exceeds the consumption during the production under suitable conditions of very considerable retinal "fatigue." The explanation, therefore, must be sought among the physiological rather than the photo-chemical conditions of the problem.

We may consider a few examples. The following phenomenon is instructive, and probably familiar to most people. On a rather misty afternoon, on coming to the window, the eyes rest on a bright gap in the clouds. At first it appears too bright to look at, and the after-image is very black. But if we persist, after a few seconds the sun's disc appears sharply defined in the midst of the dazzling light.

The retina can hardly be regarded as fatigued in the sense of being exhausted and having its activities impaired, since we may continue watching until the eyes have become completely adapted to the more brilliant illumination, and further details—spots on the sun—isolated wisps of cloud in the clear space—become visible, which were at first hidden in the glare. In the subdued light of the room the store n of sensitive material had become large.

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* 'Roy. Soc. Proc.,' vol. 69, p. 129.

On first glancing at the cloud gap the rate dx/dt at which exciting substances were formed was greater than that dn/dt at which sensitive material could be secreted. Accordingly, after a short time of almost painful brilliancy, the store n was reduced to zero, and a hand-to-mouth condition of things set in, during which $dx/dt = dn/dt$, *i.e.* just as much exciting substance was formed as could be furnished by the sensitive material secreted. But, during the interval, dx/dt was so far in excess of the normal that maximum sensation was produced both by the light of the clouds and by the sun itself. And it can hardly be denied that during the so-called retinal fatigue, after the details became visible, a condition of very great activity existed.* On the other hand, the sensation was undoubtedly less, and became less as the eyes got accustomed to it.

Similar phenomena may be seen in a furnace—a blinding glare in which details of flame and of molten metal with slag floating on it gradually appear —or with far less intensity of illumination in the "faces in the fire" in the hot coals of an open grate.

There is at first just the same sense of being dazzled—the same gradual perception of details, and in the end the same quiet contemplation of what has become comfortably visible, whether the experiment is made with the hot coals of the open grate or the far greater intensity of the evening sun. Yet the actual intrinsic luminosity of the hot coals is a good deal less than that of the newspaper which we read out of doors in the sunshine on a summer's day.

The name retinal fatigue for this state is not very apt. It is a condition in which a powerful stimulus produces a reduced effect, not because the organ is in any way deteriorated or used up, but because it works best in that way. It suggests strongly the use of a shunt with a galvanometer. And if we add the idea that with the eye it takes some little time to put the shunt on, and still longer to take it off again, we have a fairly accurate description of the facts.

The condition in which a shunt factor too strong for the exciting light persists is called the negative after-effect. Thus, if S_t = the shunt-factor at time t,

$$\frac{de/dt}{S_t} = \text{the strength of the } \textit{sensation} \text{ at that moment.}$$

This view of the negative after-effect agrees with the theory of Fechner on the subject, as described by Helmholtz.† I have been unable to consult

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* *Cf.* Waller, 'Phil. Trans.,' 1897, B, vol. 188, p. 65, note, "The retina resembles nerve with respect to its inexhaustibility."

† Helmholtz, 'Handbuch der Physiologischen Optik,' 2nd ed., p. 534.

Fechner's original papers, and do not know what was his opinion on the point, but I consider that there is strong evidence that the shunt-factor is a function of the retina rather than of the brain.

The form of the curves of colour-sensation during very bright light may now be discussed. The supply dn/dt of sensitive material is limited by the rate of metabolism possible to the tissues, but the rate at which it is converted into exciting substances, dx/dt, depends on the intensity of the light. Therefore long exposure to a bright light will make the concentration of the sensitive material, and consequently also the molecular resistance, tend to a minimum. But when friction is low the amplitude of the forced vibration is slightly greater whatever be the period of the impressed vibration, and very much greater as this approaches the period natural to the resonator, so that the curve develops a very sharp central spike. This spike cannot be reproduced in the corresponding curve of colour-sensation because of the separation of the opto-chemical from the physiological functions of the eye. The active material, however concentrated, cannot do more than excite a maximal response.

But the excess of active material may excite the protective arrangement, or shunt function, to stronger action, so that the maximal response may only send through to the central organ a quite moderate sensation.

This state of things is illustrated in fig. 3, where the dotted line represents the first beginnings of artificial green-blindness, before the slit has been opened wide enough to dazzle the eye. The response is well within the limits of possible sensation, so that there is no truncation of the curve of resonance, although by the shunt-factor its ordinates are reduced to one quarter of their normal value.

It will be noted that the effect is to lessen the apparent extent of the green sensation. And this is precisely what happens. The red sensation on the one side, and the blue sensation on the other, encroach on the green. A powerful light is not required to show this. My method of testing for colour-blindness is based upon it. After looking at the *b*-lines in a spectrum of quite ordinary intensity for 30 seconds the boundary between red and green is found to have shifted from 100 to 400 Å.U. nearer the green, if the observer possesses a normal green sensation.

The height of the apex is inversely proportional to the coefficient of friction. When, therefore, this is further reduced by the action of a strong light, a stage is reached when the curve is truncated. For the height of the apex of the resonance curve is limited because in the first place dx/dt, the rate of production of the exciting substance, cannot exceed dn/dt, the rate of secretion of the sensitive visual material, and in the second place because de/dt, the strength of the response, reaches its maximum under the action of smaller quantities of the exciting substance. Consequently the strength of the resulting sensation, when reduced by the shunt factor, will be represented by a more or less flat-topped curve.

From various data I judge that in artificial colour-blindness of quite moderate degreee, a shunt ratio of 1 : 100 before and after the exposure of the eye to light is well within the mark. I described in 1897 an experiment bearing upon this subject which seems to have escaped notice.*

When the spectrum is viewed by intermittent light of great intensity, the flash ratio being 1 : 3 or 1 : 4, as soon as the eye gets accustomed to the strong light it is seen that the continuity of the colours is gone, and that there are now visible four bands of strong colour, viz., red, green, blue, and violet, upon a pale but brightly illuminated ground. Between the intense red and the rich green is a space of a colour between yellow-ochre and raw sienna, but very pale. Between the green and the bright blue is a region of pale greenish-blue, passing into pale steel-blue. Beyond the bright blue is a pale lilac space. Farther than this cannot be seen while the red end of the spectrum is in the field, because of the overpowering intensity of the light in the neighbourhood of the yellow-green. But by shifting the prisms so that the *b*-lines come on the extreme left, while the G-line occupies the middle of the field, the region about H and K appears of a deep rich violet of great intensity, contrasting strongly with the pale lilac between it and the blue.

I suggest the following explanation. Owing to the periodic intervals of darkness during which the visual substance collects, the rate of formation of exciting substance is maximum over the whole range of the resonance. But the light is so strong that the shunt ratio is very high. Consequently, where two colour-sensations overlap, as in the yellow, we get the pale colour of the binary blend, and on either side, where only one colour-sensation is effective, the full rich tone proper to it.

The question as to how and by what mechanism the shunt function works, and where that mechanism is situated, is of the greatest interest and importance. I have already mentioned that I do not hold with those who refer it to the central organ. For one thing, it would add to the complexity of the optic nerve, as I have pointed out in my paper on "Areal Induction."† And, for another thing, it would leave the end organs in the retina unprotected. I should therefore look for the shunt mechanism in the retina itself.

If Charpentier's bands and Purkinje's recurrent images are to be regarded as due to spasmodic applications of the shunt factor, they would seem to indicate that it depends in some way upon contractile movements within the tissues. It would be quite easy to imagine an arrangement whereby a sensitive point might be dipped into or drawn out of a swarm of exciting molecules set free by the action of light on a surface close to it. The actual range necessary for such a movement to be effective would be extremely small, so that it might easily escape notice. Each retinal element would act independently of the rest, the protection from over-stimulation would obviously be complete, and spasmodic action could easily take place.

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* 'Journ. Physiol.,' vol. 21, p. 431.

† 'Roy. Soc. Proc.,' vol. 69, p. 125.

Areal induction, by which the illumination of one portion of the retina affects the surrounding areas, might result from secondary cross-connections between the retinal elements.

Note on the Laws of Weber and of Fechner.

The question arises whether the idea of a shunt factor, by which all sensations are reduced in the same ratio, is compatible with the received view that the light-sensation varies according to the logarithm of the stimulus.

Weber's law is the statement of an experimental fact, viz., that "the smallest perceptible difference of luminosity is a constant fraction of the whole intensity of the light." And this is nearly true, though, according to several observers, not accurately true, over a considerable range.

Fechner's law is on an entirely different footing. It is based on mathematical theory, and claims to establish a numerical scale of sensation:—"The intensity of the sensation varies as the logarithm of the stimulus."

If the sensation of light were a continuous function of one variable from subliminal threshold to maximum, there could be no two opinions as to the validity of Fechner's law when Weber's law is taken for granted. It is a simple, obvious, elementary application of the calculus. But the sensation of light is a function of at least three variables, viz., the production by light of the exciting substance, the stimulation by this of the end-organs, and the regulation by the shunt factor of the strength of the resulting sensation. Moreover, the perception of a difference between two sensations, which is necessarily implied in the statement of Weber's law, involves the judgments of the central organ, thus introducing a possible fourth variable. Under such conditions we are not warranted in ascribing to Fechner's law the cogency of a result obtained by the calculus as physicists use it.

The experiments of Ebbinghaus quoted by Helmholtz* go to prove that Fechner's law fails within a quite moderate range of intensities. My own experiments extending over the last three years confirm this view, and bring out, in addition, a point of considerable interest.

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* Helmholtz, 'Physiol. Optik,' 2nd ed., p. 392.

I have, since 1910, in the practical classes which I have conducted for Prof. Gotch, made a considerable number of people arrange from 7 to 10 strips of white paper at distances varying from $\frac{1}{2}$ metre to 2 metres from a candle in a dark room, so that viewed from a certain point they presented a series of apparently equal gradations of luminosity. The results were very instructive, and in one respect unexpected. Most of the men repeated the experiment two or three times in order to get what they considered a good result. Almost without exception the first attempt of each person showed considerably higher values for the ratios at the two ends of the series. In the majority of cases the difference was less in subsequent experiments, but it was evident that, to the unbiassed judgment, the eye is less sensitive to differences between the brightest objects visible and also between the faintest objects visible at any one time than between those that are moderately illuminated.

Although practice reduces this divergence from Weber's law it does not do away with it. I still make the ratios at the two ends of the series higher than those near the middle of it. But with monochromatic red light any series that looks right to me under a feeble illumination looks right also in light 100 times as strong, as it should do according to Weber's law.

I am inclined therefore to accept Fechner's law with the same caution that Waller expressed in reference to the relation between strength of stimulus and the retinal currents of the frog's eye. "The curve plotted from the data comes out concave towards the abscissæ and not unlike an ordinary logarithmic curve."

If, in judging the minimum perceptible difference of brightness, we instinctively make use of the shunt function only, that would lead to something not far removed from the logarithmic law, within the range covered by the shunt.

The greater part of the experimental work connected with this paper has been done in the Physiological Laboratory, Oxford, and the expenses have been defrayed out of the Government Grant Fund.

The Various Inclinations of the Electrical Axis of the Human Heart. Part I.—*The Normal Heart.*

By A. D. Waller, M.D., F.R.S.

(Received March 6,—Read May 8, 1913.)

(From the Physiological Laboratory of the University of London, South Kensington.)

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1. *Introductory—Weak and Strong Leads.*

The "normal hearts" dealt with in the present communication are those of the regular workers in this laboratory and of ordinary visitors who have wished to see the electrical effects of their own hearts and who have been good enough to allow me to make use of their records; these have been taken when possible by right and left superior and by right and left lateral leads, so as to afford data for the calculation of the position of the current-axis above and below the heart. No clinical examination of any kind was made in the case of visitors, so that strictly speaking normality of the heart in their case has not been verified. We may, however, assume as probable that the heart of an ordinary active person does not depart widely from the normal. And as a matter of fact the electrocardiograms taken upon such persons are in themselves sufficient evidence of normality to an observer familiar with the signs of abnormality.

In the entire series of normal persons (amounting to about 200) I have examined during the last three years I have only met with five cases presenting "abnormal" cardiograms; of these five, three have volunteered an interest in their own cases that has permitted a careful examination of the heart to be made, in the other two cases no attempt at verification has been made or suggested.

One of the "normal" cases has proved to be particularly interesting, that namely of Thomas Goswell, my former laboratory man, upon whom I made repeated observations during the year 1887 and whose current-axis I then estimated, or rather guessed, as forming an angle of 45° with the vertical line. His normality was, of course, verified at the time. And I have been fortunate enough a few days ago to obtain records of the four necessary leads from which the angle of the current-axis can be calculated. It happens to come out at the value of 45°, which in itself is, in my opinion, proof of normality. And the photograph that was taken of this subject last week as compared with the sketch of the same subject that was used as a class-diagram 27 years ago is in itself sufficient proof of normality as to the state of the heart.

In 1887 the idea occurred to me that it should be possible to utilise the limbs as natural electrodes in relation with more or less opposed aspects of the heart, and so to obtain information concerning the absolutely intact organ. With the aid of Lippmann's capillary electrometer I surveyed all the different pairs of leads that I could think of, and as the outcome of this survey, divided the human body into two unequal parts by means of an imaginary line or equator cutting at right angles a second imaginary line or current-axis crossing the chest obliquely in the direction of the anatomical axis of the heart—itself an imaginary or at least an indefinite line. I figured the current-axis as forming an angle of 45° with the vertical and taking the leads two by two I found that they fell into two sets which I called "favourable" and "unfavourable." On review of these observations it became apparent that in the "favourable" cases, *i.e.* those in which the electric pulse was obvious, the two leads were on opposite sides of the equator, while in the "unfavourable" cases, *i.e.* those in which little or no pulse was visible, both leads were on the same side of the equator. I ascertained by direct observation that of the six possible leads afforded by the four extremities taken two by two, three are favourable (1, 2, 3) and three unfavourable (4, 5, 6) as regards the demonstration of the electrical pulse; and that of the four possible leads from the extremities taken in conjunction with the mouth, three are favourable (7, 8, 9) and only one unfavourable (10).*

Finally the proof of the relation between the normal obliquity of the heart and favourable and unfavourable leads was completed by the investigation of two cases of situs viscerum inversus where in correspondence with the reversed obliquity of axis, the transverse effect between the two hands was observed to be reversed; the right superior and left lateral leads to be favourable; the left superior and right lateral to be unfavourable.

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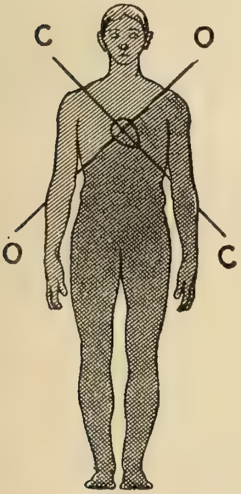
* Waller, "On the Electromotive Changes connected with the Beat of the Mammalian Heart, and of the Human Heart in particular," 'Phil. Trans.,' 1889, p. 169. The principal facts were demonstrated, in 1887, at the First Congress of Physiology, at Bâle.

The facts have been confirmed by all subsequent observers, more or less completely according as they have reviewed more or fewer of the 10 leads, but with one notable exception, viz., the left lateral, as to which I am stated to have been mistaken. Prof. Einthoven in particular has attributed my having classified it as an "unfavourable" or "weak" lead to the comparative slowness of the capillary electrometer;* as a matter of fact I did not make the mistake attributed to me, and I expressly adhere now to my original classification of the left-hand lead with either foot as a "weak" lead. The classification of leads as "strong" and "weak" forms indeed the basis of this paper, in the course of which it will be made apparent that the relatively weak left-hand effect below the heart is an index of the degree of obliquity of the cardiac current-axis.



1. Transverse...	R. hand.	L. hand.
2. Axial.........	R. hand.	L. foot.
3. R. lateral ...	R. hand.	R. foot.
4. L. lateral. ...	L. hand.	L. foot.
5. Equatorial...	L. hand.	R. foot.
6. Inferior	R. foot.	L. foot.
7. L. superior ..	Mouth.	L. hand.
8. L. inferior ...	Mouth.	L. foot.
9. R. inferior...	Mouth.	R. foot.
10. R. superior ..	Mouth.	R. hand.

C C denotes the current-axis, O O denotes the equator.

When the survey of these ten leads has been completed, we may reduce them in number for further systematic investigation. The two feet are practically iso-electric: this pair may therefore be neglected. And if we regard the two feet as electrically indifferent, the axial and right lateral leads are equivalent, and one of them may be left out; also, on this assumption, the left lateral and the equatorial are equivalent, and we may drop one out. Then as to the mouth; it is not necessary in a cursory survey to take all the three "favourable" leads: it is sufficient to compare the "good" hand (left) with the "bad" hand (right) by taking the right and left superior leads. And so I reduce my system of leads to five, as under:—

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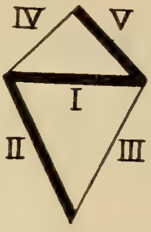
* Einthoven ('Pflüger's Archiv,' 1908, pp. 551–2). In my first three cases of 1887 the angles (measured in 1913) are 45°, 51°, and 86°. In Einthoven's two cases of 1908 (measured from the published records on pp. 554–5) the angles are—Ei. = 22° and Fl. = 7°.

I. Transverse.
II. Right lateral.
III. Left lateral.
IV. Right superior.
V. Left superior.



Note.—In adopting this simplification we should, however, not lose sight of the fact that, for certain finer determinations, the P.D. between the two feet must be taken into reckoning, and that we may not always take as equivalent axial with right lateral and equatorial with left lateral, although for all ordinary purposes this may be done. Also, in the case of the mouth leads, where we shall content ourselves with taking the left superior as the strong lead and neglect the other two strong leads, viz., left and right inferior, we may find it necessary to take into account the small differences that obtain between the three strong leads with the mouth. An idea of their order of magnitude is given by the following example, in which they were carefully measured:—

Right superior	3	mm.	(= 0·00023 volt)
Left superior............	15·3	„	(= 0·00118 „)
Right inferior	16·5	„	(= 0·00126 „)
Left inferior	17·5	„	(= 0·00135 „)

The study of the subject was subsequently taken up by Einthoven and his pupils, whose principal publications appeared in 1895, 1900, and 1908. In 1900 he extended the inquiry to the abnormal heart. In 1903 he devised his string-galvanometer, which, by reason of its superior rapidity, is preferable to the capillary electrometer for these observations. At the same time, Einthoven reduced the six possible leads from the extremities to three, and promulgated what is known as "Einthoven's equation," viz.:—

Lead II (axial)—Lead I (transverse) = Lead III (left lateral),

and represented in the form of an equilateral triangle, of which the heart is the centre. And quite recently* Einthoven has given a construction by which he synchronises the three records, in order to calculate from their corrected value the magnitude of the angle α that must be formed with the horizontal by a potential difference passing through the centre of the triangle. From this basis Einthoven has calculated that in a particular subject ("Bak") the heart had rotated in the chest around a sagittal axis during the movement of expiration by an angle of $-36°$ (*i.e.* from $\alpha = 76°$ to $\alpha = 40°$, referred to the horizontal).

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* W. Einthoven, "Ueber die Form des menschlichen Elektrocardiogramms," 'Pflüger's Archiv,' 1895, vol. 60, p. 101; W. Einthoven and K. de Lint, "Über das normale menschliche Elektrocardiogramm, und über die capillar-elektrometrische Untersuchung einiger Herz-Kranken," 'Pflüger's Archiv,' 1900, vol. 80, p. 139; W. Einthoven, "Die

2. *Determination of the Electrical Axis.*

I have approached this problem of the angle from a different standpoint, namely, from the point of view of my first observations of 1887–9, and the distinction between "favourable" and "unfavourable" leads, or, as I now call them, "strong" leads and "weak" leads. In order to calculate the obliquity of the cardiac current-axis, I took values between a mesial point and the two sides of the body, in fulfilment of the rough notion of a balance of which the two unequally loaded arms, R and L, give an angular deflection of the indicator in relation with a weight-difference between R and L.

In electrical analogy with this idea, I took for calculation the values of the potential differences between leads from the mouth and right hand and between the mouth and left hand. According to this picture, the electrical pivot or zero is an electrode in the mouth, and the weights are the potential differences between the electrode M and the right hand R on one side, and the left hand L on the other, to form the other electrode. These two leads may be referred to as the right superior and left superior respectively, in distinction from analogous leads between hands and feet, which will be referred to as right and left lateral. The formula for calculating the angle α between current-axis and vertical is very simple: $\tan \alpha = (L-R)/(L+R)$, where L and R represent respectively observed magnitudes of potential difference at the outset of systole by the left and right superior leads respectively; for example, with $L = 9$ and $R = 3$,

$$\tan \alpha = \frac{9-3}{9+3} = \frac{6}{12} = 0{\cdot}5, \qquad \therefore \quad \alpha = 27°.^*$$

Similar considerations apply to the inferior (or posterior) half of the body, the feet (or either foot if we admit that the small P.D. existing between the

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galvanometrische Registrirung des menschlichen Elektrocardiogrammes, zugleich eine Beurtheilung der Anwendung des Capillar-Elektrometers in der Physiologie," 'Pflüger's Archiv,' 1903, vol. 99, p. 472; "Le Télécardiogramme," 'Archives Internat. de Physiol.,' 1906, vol. 4, p. 132; "Weiteres über das Elektrocardiogramm," 'Pflüger's Archiv,' 1908, vol. 22, p. 517; "The different Forms of the Human Electrocardiogram and their Signification," 'Lancet,' March, 1912, p. 853.

* Or, more precisely, 26° 34′ but in this connection α will be given to the nearest degree only.

two feet may be treated as negligible; but by reason of the greater acuteness of the angle subtended by the R and L leads at the inferior lead F, as compared with the superior lead M, a new factor must be introduced into the formula, which is now taken as $\tan\alpha = 2(R-L)/(R+L)$, where R and L represent respectively observed magnitudes of the potential differences in the right and left lateral leads. If, for example, the values have been observed, $R = 12$, $L = 4$, then

$$\tan\alpha = 2\frac{12-4}{12+4} = \frac{16}{16} = 1, \qquad \therefore \quad \alpha = 45^\circ.$$

Put into words, the general conclusion expressed by the two formulæ* for calculating the direction of the cardiac current-axis from the right and left potential difference existing at the outset of systole above and below the heart, is as follows:—The tangent of the angle formed by the current-axis with the vertical is proportional to the difference between the potential differences of the strong lead and the weak lead divided by the sum of these two differences.

The error of angle by error of measurement of the spike is, with most records, inconsiderable, except in the case of the left lateral lead, when its direction is doubtful. When it consists of a small positive followed by a large negative peak we may hesitate whether to take into our formula the small positive or the large negative value; in such case of doubt it is advisable to calculate the angle for both values. If, in a doubtful case, the right lateral is smaller than the transverse spike, the negative value of the left lateral should be taken in the formula; if the right lateral is larger than the transverse, the positive value of the left lateral should be taken. But I do not attach much value to the numerical result in such a case (*vide infra* fig. on p. 520, the case of Dr. E.).

In most normal records, where the ventricular spike is clearly positive, there is no difficulty in measuring out its values for the right and left hands. I have not taken into reckoning the small preliminary negative movement ("Q") by which the positive movement is more or less distinctly preceded. This negative movement, while forming part of the systolic change of potential, occurs before the ventricle has begun to contract.

As far as it is possible to judge from simultaneous records by the R and L lateral leads, taken on a film travelling at the rate of 130 mm. per second, the two spikes R and L, when both are positive, culminate synchronously.

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* The two formulæ might have been expressed by a single general formula, as follows: $\tan = \cot\theta(S-W)/(S+W)$, where θ stands for the angle taken at M or at F, and S, W for the values of strong and weak leads respectively. But the two special formulæ are preferable for practical calculations with a conventional direction of currents.

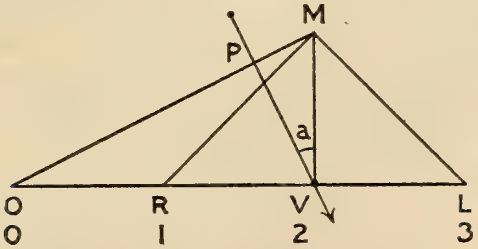
Where the spike is positive on the right side and negative on the left, the positive maximum was reached 0·006 sec. earlier than the negative minimum, and, as far as can be judged from the record, the two events appear to commence synchronously. From the inspection of the left lateral spikes of intermediate type, + −, it appears that the transition from 1 to 4 is by a retrograde encroachment of − upon +.

3. *Proof of the Formulæ :* $\tan\alpha = \frac{L-R}{L+R}$, $\tan\alpha = 2\frac{R-L}{R+L}$.

The normal difference between "strong" and "weak" leads is evidently dependent upon the normal obliquity of the heart. The principal electrical event of the beat, *i.e.* the systolic spike, is the resultant along a line BA or CC representing the current-axis, of all the component differences of potential existing in the heart muscle at the outset of systole. Comparing the magnitudes of the spikes of the two sides it is obviously in correspondence with the normal tilt of the heart's axis that the left superior is the "strong" lead and the right superior the "weak" lead.

From the observed magnitude of the two spikes, right and left, it is easy to construct a geometrical figure by means of which the numerical value of the obliquity can be expressed in terms of the angle made by the current axis CC with the vertical line MV.

In the triangle MRL, the sides MR, ML indicate the right and left superior leads, M being the mouth, R the right hand, L the left hand. Actually, in relation to the heart, these leading points are to be regarded as sections through the neck and shoulders, and for convenience of calculation we shall take the angle at $M = 90°$, so that $MV = \frac{1}{2} RL = RV$. Taking, *e.g.* the right-hand spike = 1 and the left-hand spike = 3, we have



at M the potential = 0, at R the potential = 1, at L the potential = 3. Projecting the potential of M on to the horizontal line LR produced to O and taking the position of the point O such that the length OR shall represent the potential difference between M and R, and OL the potential difference between M and L, we have MO as the line of zero potential. A line VP, drawn perpendicular to this zero line MO, gives the position of the current-axis CC forming with the vertical MV an angle, which call α.

The angle MOV $= \alpha$; $\tan\alpha = \tan \text{MOV} = \text{MV}/\text{OV} = \frac{1}{2} = 0{\cdot}5$. Therefore $\alpha = 26° \, 36'$; or otherwise: since $\text{MV} = \frac{1}{2}(\text{L}-\text{R})$ and $\text{OV} = \frac{1}{2}(\text{L}+\text{R})$, we may write $\tan\alpha = (\text{L}-\text{R})/(\text{L}+\text{R})$, or, in words, the required angle α is an angle having for its tangent the fraction of which the numerator is the difference between the spike of the strong lead and that of the weak lead, and the denominator their sum.

The formula holds good for negative values of the weak lead, as can easily be shown by a geometrical construction. But an example will be sufficient. Let -1 be the value observed by the weak lead R, and $+3$ that for the strong lead L.

$$\tan\alpha = \frac{\text{L}-\text{R}}{\text{L}+\text{R}} = \frac{3+1}{3-1} = 2, \qquad \therefore\ \alpha = 64°.$$

Finally, we may mention two particular cases that are occasionally observed. If the weak lead $\text{R} = 0$, then

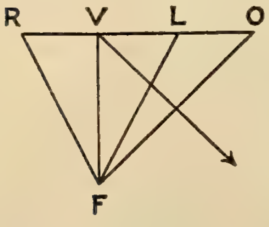
$$\tan\alpha = \frac{\text{L}-0}{\text{L}+0} = 1, \qquad \therefore\ \alpha = 45°.$$

If the weak lead R is negative and greater than the strong lead we shall have α greater than 90°, *i.e.* a current-axis upwards from right to left. This case may also occasionally be met with as an extreme case of the "cor breve et molle," *e.g.* let $\text{R} = -2$ and $\text{L} = 1$,

$$\tan\alpha = \frac{\text{L}-\text{R}}{\text{L}+\text{R}} = \frac{1+2}{1-2} = \frac{2}{-1} = -2, \qquad \therefore\ \alpha = 116°.$$

An essentially similar principle of calculation is applicable to the data afforded by leads from the four extremities—the two hands and the two feet—with, however, certain modifications.

The modifications to be taken into account are: (1) that the two feet are assumed to be equipotential and represented by a single foot F at the inferior angle of the triangle RLF in analogy with the point M of our first triangle; (2) that therefore we are to regard as equivalent the right lateral with the axial leads, and the left lateral with the equatorial; (3) that we have to remember that with the extremities, R is the strong lead, L the weak lead; and (4) that the conditions require us to take at F a more acute angle than in the case of the superior triangle we have taken at M.



In the figure representing the inferior triangle the length VF has been taken as equal to the length RL, *i.e.* twice RV. The formula now runs: $\tan\alpha = 2(\text{R}-\text{L})/(\text{R}+\text{L})$, where R and L stand for the magnitudes of the systolic spikes of the right lateral and left lateral leads respectively. (In the illustration we have taken R = 3 and L = 1.)

It is scarcely necessary to give the geometrical construction from which the formula is derived. As in the previous case we have tan α = the difference R−L divided by the sum R+L and multiplied by FV/RV, the cotangent of $\frac{1}{2}$ the angle RFL. For the superior triangle we took MV/RV = 1, for the inferior angle we take FV/RV = 2.

The application of our formula may be illustrated by examples. It has been observed, *e.g.*, that R = 3 and L = 1; then

$$\tan\alpha = 2\frac{3-1}{3+1} = \frac{4}{4} = 1, \qquad \therefore \quad \alpha = 45^\circ.$$

It has been observed, *e.g.*, that R = 3 and L = −1; then

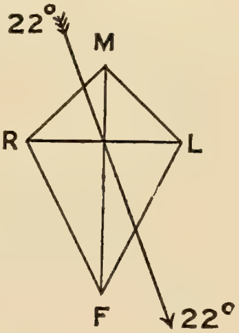
$$\tan\alpha = 2\frac{3+1}{3-1} = \frac{8}{2} = 4, \qquad \therefore \quad \alpha = 76^\circ.$$

We shall now apply our formula to some actual cases:—

The Case of B. O. B.

(Inspiratory Values.)

Right superior	7·5
Left superior	17·5
(Transverse	16)
Right lateral	23·5
Left lateral	15·5

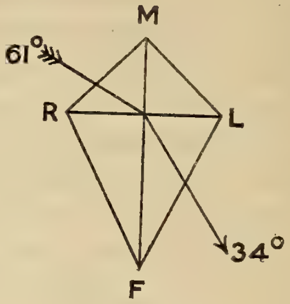


$$\textit{Sup.} \quad \tan a = \frac{L-R}{L+R} = \frac{17{\cdot}5-7{\cdot}5}{17{\cdot}5+7{\cdot}5} = \frac{10}{25} = 0{\cdot}40, \qquad \therefore \quad a = 22^\circ.$$

$$\textit{Inf.} \quad \tan a = \frac{R-L}{R+L} = 2\frac{23{\cdot}5-15{\cdot}5}{23{\cdot}5+15{\cdot}5} = \frac{16}{39} = 0{\cdot}41, \qquad \therefore \quad a = 22^\circ.$$

The Case of J. C. W.

Right superior	−2
Left superior	7
(Transverse	11)
Right lateral	14
Left lateral	7

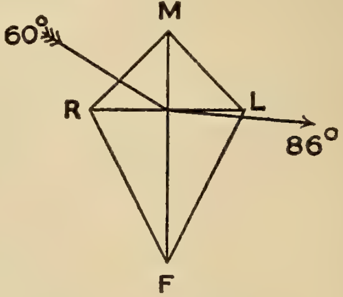


$$\textit{Sup.}\quad \tan\alpha = \frac{7+2}{7-2} = \frac{9}{5} = 1{\cdot}8, \qquad \therefore\ \alpha = 61^\circ.$$

$$\textit{Inf.}\quad \tan\alpha = 2\,\frac{14-7}{14+7} = \frac{2}{3} = 0{\cdot}67, \qquad \therefore\ \alpha = 34^\circ.$$

The Case of A. D. W.

Right superior	−2
Left superior	7·5
(Transverse	12·5)
Right lateral	10
Left lateral	7·5



$$\textit{Sup.}\quad \tan\alpha = \frac{7{\cdot}5+2}{7{\cdot}5-2} = \frac{9{\cdot}5}{5{\cdot}5} = 1{\cdot}73, \qquad \therefore\ \alpha = 60^\circ.$$

$$\textit{Inf.}\quad \tan\alpha = 2\,\frac{10+7{\cdot}5}{10-7{\cdot}5} = \frac{35}{2{\cdot}5} = 14. \qquad \therefore\ \alpha = 86^\circ.$$

4. *Apparatus Used.*

In these observations I have used:—

(1) An early model by Edelmann of Einthoven's galvanometer with a platinum fibre of 2000 ohms resistance, and platinum electrodes dipping in normal saline.

(2) An oscillograph (Bock-Thoma model) by Thoma, of Munich, with a condenser of large capacity (10 and 20 mf.) in circuit, and, as before, platinum electrodes in saline.

Each of these instruments possesses advantages and disadvantages; for the special purposes of the present observations the second instrument has proved to be the more convenient. By the use of platinum electrodes (which in themselves act as a condenser of considerable polarisation capacity, *e.g.* in one measured case about 10 mf.), supplemented by an added condenser in circuit, we are rendered independent of alterations of resistance; the galvanometer is practically converted into an electrometer.

5. *Influence of Position and of Respiration.*

The influence of slight alterations of position of the body upon the electrical record both as regards form and as regards amplitude is trifling. I did not notice any such influence in my first observations. Einthoven has subsequently found that in the recumbent posture turning from the left over to the right side alters the form of the first ventricular wave from simply + to + −. But in view of the calculations to be made from the relative amplitudes of the left and right records, I thought it necessary to re-try this point in order to learn whether alterations of position great or slight cause alterations of amplitude. I found that slight alterations are negligible, but that great alterations, such as from standing to sitting, and lying either on the back or on the face, or on one or other side, alter the amplitude and the angle. I have therefore taken all observations from persons in the most convenient position, *i.e.* sitting.

The effect of considerable alterations of position is evident from the following observation, in which the transverse, right and left lateral records were taken of the subject B. O. B. in the standing, sitting, and lying positions. The points that come out most clearly on review of this group of records are that lying on the left side as compared with lying on the right side diminishes the angle α, as shown by increase of the left lateral spike and diminution of the transverse spike. This alteration is also brought about by muscular exercise (*vide infra*), and the reverse alteration, viz., increase of the angle α, is caused by distension of the stomach.

B. O. B. (Feb. 26, 1913).

	Pulse frequency.	Transverse.	R. lat.	L. lat.	α.*
					Insp. Exp.
Standing	74	14	14	7·5	31 (29 45)
Sitting	64	15	14	5	43 (40 53)
Lying on back	62	15	18	7·5	40 (39 50)
Lying on right side...	62	17	18	5	48 (42 50)
Lying on left side ...	60	11	16	10	25 (25 27)

* The values of α given in parentheses are the *approximate* inspiratory and expiratory numbers calculated from maximal and minimal values of the R and L spikes.

There is no more than a general correspondence of inclination between the line drawn through the heart to represent its anatomical axis and the line or lines representing its electrical axis. This is hardly surprising when we realise how indefinite is the anatomical axis and upon what data and assumptions the determination of the electrical axis depends. I regard the latter as the more definite of the two lines; it gives expression to the functional resultant at the outset of contraction of the living organ, and indicates by considerable and definite variations changes of axis that are difficult to settle either *post mortem* or by skiagram during life.

6. *Tone of the Heart Muscle.*

I attribute considerable importance to the tone of the heart muscle as regards position of the heart and of its electrical axis. With soft muscle the heart is sessile on the diaphragm, and the axis, as calculated from right and left lateral spikes, is approximately horizontal. (See fig. on p. 520, the case of Dr. E.) With hard muscle the heart is more nearly erect on the diaphragm, and the axis, calculated as before, is more nearly vertical. (See fig. on p. 521, the case of Dr. D.)

Influence of Respiration.—I paid no attention in my first observations to the effect of respiration upon the electrocardiogram. This effect has since been carefully studied by Einthoven, who has shown that with forced inspiration the amplitude of the record of Lead III (left lateral) is increased, while that of Lead I (transverse) is diminished; the changes in Lead II (axial) he describes as very slight. My observations are on the whole in harmony with those of Einthoven, but their full discussion must be postponed. For the purpose of the present communication it will be sufficient to state that I have found it necessary for any exact estimation of the angle to take into account the phases of ordinary respiration, which briefly are of the following character:—

With inspiration the left superior and the right lateral records are diminished, the right superior and the left lateral records are increased. In taking out values of R and L for a careful determination of α by the appropriate formulæ it is therefore necessary to take for its inspiratory value the smallest values recorded of the left superior and right lateral leads and the largest values recorded of the corresponding right superior and left lateral leads. For the expiratory value we must take out the largest values of the left superior and right lateral and the smallest values of the right superior and left lateral. But this troublesome correction is in most cases superfluous. It can, however, occur that normal respiration brings out differences of amplitude that lead to differences of angle of 10°, *e.g.* the case of J. B. F., where I first noticed that the correction might be required, and I have therefore thought necessary to say that I have been alive to the error that might be made by neglecting the correction where it was evidently of moment.

The type of the electrical pulse by different leads is remarkably individual and constant. Thus it has not altered, as far as I can tell, in the cases of A. D. W. and A. M. W. and T. Goswell between 1887 and 1913.

Nevertheless, temporary variations do occur in a given individual—variations of frequency of course, but also variations of form and variations of the angle α. I think that such variations are attributable to greater and lesser repletion of the stomach and of the several cavities or of the two sides of the heart. In at least two instances where the angle α has been found greater in the same individual at one time than at another, I have associated the value of the angle with the state of health. But the discussion of this important point cannot profitably be entered upon until the effect of respiration has been fully considered; and it properly belongs to a future communication on the pathological significance of the angle α.*

7. *The Influence of Muscular Exercise.*

As was to be expected, the frequency and character of the electrical pulse are altered with muscular exertion.

Electrocardiograms afford indeed the most convenient available means of exactly counting the pulse, and of measuring out the working-time of the heart from varying relations between length of systole and length of diastole. But the primary object of this paper is to study variations of the angle α, and in this connection it appears that any variations that might be expected to result from variations of repletion of the several cavities is masked by the large variations caused by deepened respiration—especially inspiration. The detailed consideration of the influence of exercise must therefore be subordinated to that of the influence of respiration, and at present it will be sufficient to give the results of observation on one subject (B. O. B.) in illustration of the fact that muscular exertion indirectly through modified respiration, and perhaps also directly by modifying the repletion and shape of the heart, does actually bring about considerable modifications of the electrocardiogram and of the angle α as calculated from its right and left hand values.

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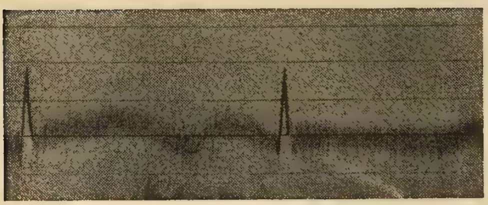
* A further complication arises where the left lateral lead is negative; the correction has then to be taken in the opposite sense, because whereas a positive left lateral is increased with inspiration, a negative left lateral is diminished. The same holds good for a negative right superior record. But the discussion of these points must be postponed.

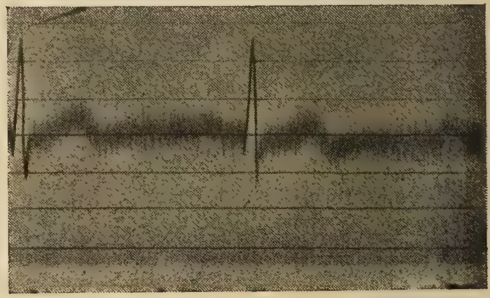
Dr. E.—The superior angle a can be estimated without difficulty from the values of the right and left superior spikes—

$$\tan a = \frac{12{\cdot}5+7{\cdot}5}{12{\cdot}5-7{\cdot}5} = \frac{20}{5} = 4. \quad \therefore\ a = 76^\circ.$$

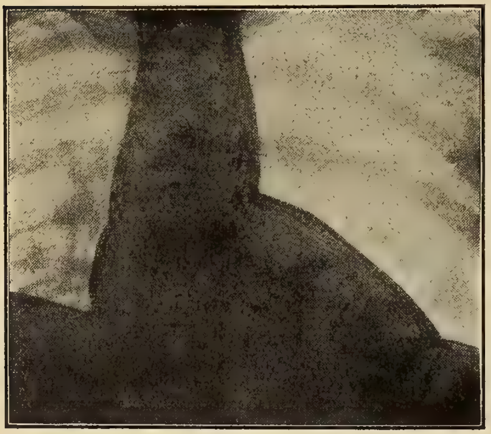
But the mixed character of the right and left lateral spikes, viz., positive followed by negative, does not afford assured data for calculation (see text, p. 512). Taking into the formula the positive values 7·5 and 5, the angle comes out as 22°. Taking a positive value 7·5 and a negative value -25, $a = 105^\circ$. Taking the negative values $-12{\cdot}5$ and -25, $a = 34^\circ$ of reversed current direction. But I place no reliance on these figures.

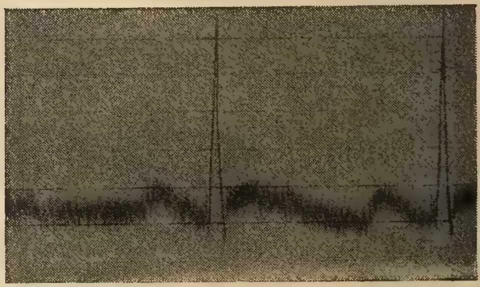


Right superior.

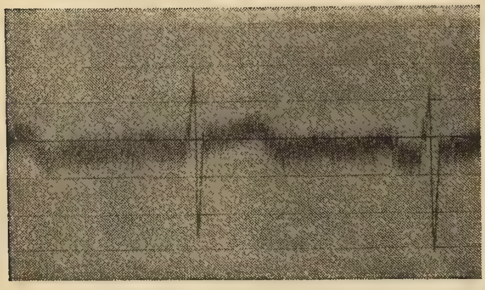


Left superior.

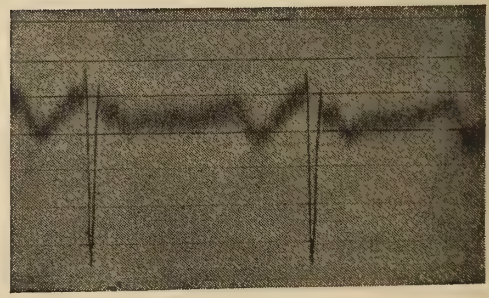




Transverse.



Right lateral.

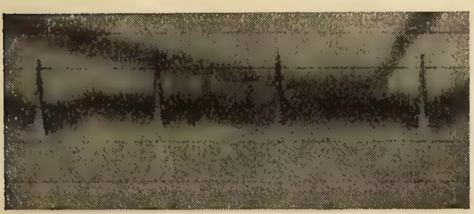


Left lateral.

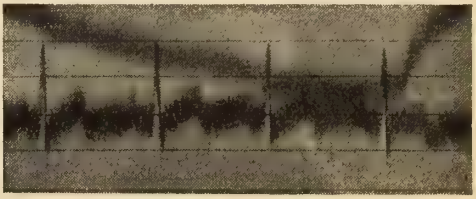
Dr. D.—In this case the electrocardiograms were taken with a simultaneous record of the respiratory movements, and the measurements are given for the same (inspiratory) position in each case. Upon another occasion the R. and L. values were found to be 20 and 15, *i.e.* the inferior α came out = 16°, but no attention was then paid to the phase of respiration. In the accompanying records the values are as under :—

$$\textit{Sup.} \quad \tan\alpha = \frac{7{\cdot}5-5}{7{\cdot}5+5} = 0{\cdot}2. \quad \therefore\ \alpha = 11^\circ.$$

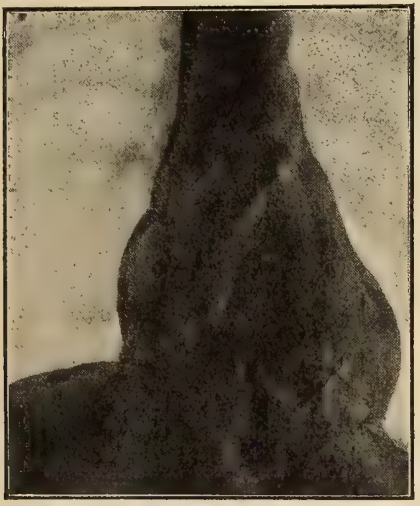
$$\textit{Inf.} \quad \tan\alpha = 2\,\frac{22{\cdot}5-20}{22{\cdot}5+20} = 0{\cdot}12. \quad \therefore\ \alpha = 7^\circ.$$

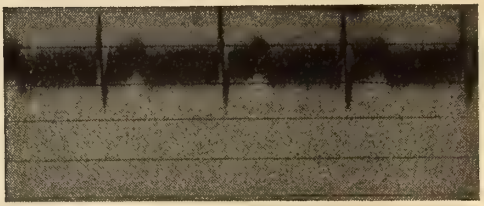


Right superior.

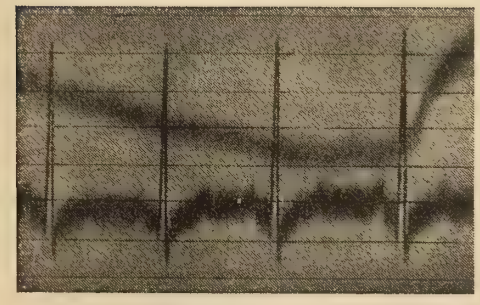


Left superior.

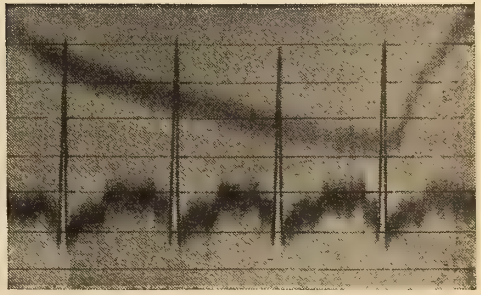




Transverse.



Right lateral.



Left lateral.

	Pulse frequency.	Transverse.		R. lat.		L. lat.		α.	
		Insp.	Exp.	Insp.	Exp.	Insp.	Exp.	Insp.	Exp.
At rest	66	10	11	13	15	7	5	31°	45°
Immediately after exertion, 2000 kgrm. in 35 secs.......	146–126	7·5	12·5	12	14	10	5	10°	44°
Two or three minutes later...	98–96	10	15	13	15	9	5	20°	45°

	Pulse frequency.	R.	L.	α.
Normal	96 to 86	11	2·5	52°
			Exp. Insp.	Exp. Insp.
Second minute	120	12·5	5 to 10	41° to 12°
Fifth minute	104	14	3 ,, 5	52° ,, 43°
Thirtieth minute ...	72	12·5	2·5	53°

The inferior angle α is diminished in consequence of muscular exertion.

8. *The Influence of Food.*

From measurements taken on the same individual before and after food, the angle α has come out greater in the latter than in the former state. But the numerical estimation of this difference cannot be discussed with profit apart from the consideration of the respiratory variations of angle. In the case of B. O. B. the difference has come out = 10°, the actual measurements having been as under:—

	Pulse frequency.	Transverse.	R. lat.	L. lat.	tan α.	α.
Before dinner	62	11	12·5	6	0·70	35°
After dinner	70	12·5	12·5	4	1·02	45°

The left lateral is smaller with a full stomach.
The right lateral is not appreciably altered.
The transverse is increased.
The angle α is increased.

9. *Thoracic Leads.*

The preceding considerations dealing with leads from the mouth and extremities apply to the current-axis in the frontal plane. Similar considerations can be applied for measurement of the current-axis in the antero-posterior (sagittal) plane, and we may calculate superior and inferior values of α by the same formulæ as those taken for their values in the frontal plane.

To compare effects in the sagittal plane, the leads to be taken are:—

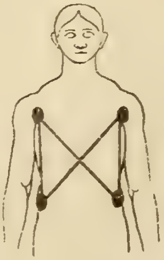
Mouth to precordium and mouth to back for the upper part of the body; back to foot and precordium to foot for the lower part. We can calculate the value of an angle α formed by the current-axis and the vertical in the antero-posterior or sagittal plane, by taking the values of the two upper and two lower spikes. And without laying stress upon the precise values obtained for the angle it is satisfactory to find that the arrow representing its position and direction comes out from the calculation conformably with what might be anticipated.

To complete our picture of the current direction we may compare effects horizontally in transverse section across the body; this can be done roughly by the leads from precordium and back to the right and left hands, or better, by taking the effects from four symmetrical leads encircling the chest on a level with the heart.

This symmetrical arrangement of electrodes gives a more satisfactory set of values than is afforded by the two hands with the front and back of the chest, which gives values in an oblique and principally frontal plane, as is illustrated by the following group of measurements:—

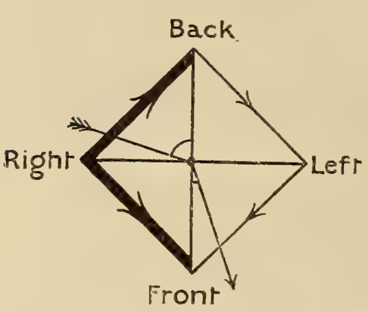
B. O. B. connected to an oscillograph by large (100 × 75 mm.) electrodes on the right and left sides of the chest, and by the right and left hands dipping in saline, gave the following values:—

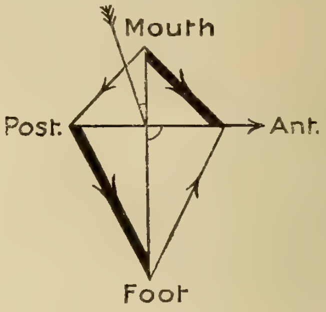
1.	Right hand Right side	+10.
2.	Left hand Left side	+7·5.
3.	Right hand Left side	+22·5.
4.	Left hand Right side	+2·5. −5.



At first sight these results appeared rather perplexing, but their obscurity disappears when we reflect that leads from the hands are, as regards the heart, equivalent to leads from the shoulders, and plan the results accordingly along the lines of a frontal diagram. We then realise that of the four leads, the 3rd, being "axial" as regards the heart, must be the "strongest" lead, and the 4th lead, being "equatorial," must be the "weakest" lead.

In a transverse section of the thorax, *i.e.* in the horizontal plane with the body in the erect posture, similar differences obtain between the two sides. Taking leads from the precordium and first the right then the left hand, we find that the first lead is stronger than the second. Taking leads from the back of the chest, and first the right then the left hand, we find that the





first lead is positive, the second small. Taking, *e.g.* the values observed for these four leads in the case of B. O. B. the angles come out as follows:—

$$\tan \alpha = \frac{16-7{\cdot}5}{16+7{\cdot}5} = 0{\cdot}36, \qquad \therefore \quad \alpha = 20^\circ,$$

$$\tan \alpha = \frac{7{\cdot}5+5}{7{\cdot}5-5} = 5, \qquad \therefore \quad \alpha = 79^\circ.$$

The angle formed above the heart by the current-axis with the vertical in this plane can be estimated from the relative values of the spike in the two leads mouth-precordium and mouth-back. Thus, *e.g.* in the subject B. O. B., the values of the spike in these leads are: anterior = 25, posterior = 10, from which our formula for the superior triangle gives $\tan \alpha = (25-10)/(25+10) = 15/35 = 0{\cdot}43$, so that the required angle $\alpha = 23^\circ$. The angle below the heart can be obtained by calculation from the values observed in the two leads precordium-foot and back-foot. In the case of B. O. B. these values are: anterior = -10, posterior = 10;

$$\tan \alpha = 2\,\frac{10+10}{10-10} = \propto, \qquad \therefore \quad \alpha = 90^\circ.$$

This result may be taken as indicating that for the superior part of the heart and body the algebraic sum of current is to be pictured as an arrow directed forwards and downwards at an angle of 23° with the vertical; while for the inferior part of the heart and body the current-axis is represented by an arrow directed horizontally forwards at an angle of 90° with the vertical.

I have tabulated, from my laboratory notes and records of "normal" subjects, the values of right and left hand records, and of the angle α calculated from them.

Values of α calculated from Workers in and Visitors to the Physiological Laboratory.

No.		Age.	Trans-verse.	Right superior.	Left superior.	α superior.	Right lateral.	Left lateral.	α inferior.
						°			°
1	Thomas Goswell	53	8	0	8	45	9	3	45
2	A. D. W.	56	12	−2	7·5	60	10	− 7·5	86
	Do.		12·5	−5	10	72	7·5	−15	108
3	A. M. W.	53		−3	8	66	13	3	47
4	A. G. W.	27	5	6	8	8	16	18	−10
5	W. W. W.	24	8·5	0	9·5	45	10	− 1·5	70
6	M. D. W.	26	11	2	10·5		6·5	− 8	93
7	J. C. W.	21	11	−2	7	61	14	7	34
8	B. O. B. .... (Insp. values)	20	14	7·5	17·5	22	23·5	15·5	22
		29					9	3	45
9	A. S.		5	0	5	45	5	+ 1 − 5	53 90
10	W. L. S.		5 8	2·5	9	29	10 10	8 5	13 33
11	J. A. G.	46	18	5	17·5	29	8	− 4	81
	Do.		18	4	16	31	Insp. 12 Exp. 16	−10 − 6	80 83
12	G. E.	29		2	7·5	30	22·5	17·5	14
13	J. S.			0	15	45	17·5	10	28
	Do.						15	7·5	33
	Do.						Exp. 20 Insp. 17	7·5 11	42 23
14	Dr. G.			5	10	18	12·5	15	−10
	Do.			5	7·5	11	10	10	0
15	Dr. R. W.		12	−2·5	7·5	64	5	2·5 −10	33 99
16	Dr. H. D.		5	5	7·5	11	20	15	16
	Do. .... (Insp. values)						22·5	20	7
17	Dr. E.			−7·5	12·5	76	7	5 −17	102
	Do.		20	−7·5	12·5	76	+ 7·5 −12·5	+ 5 −25	105
18	Dr. A.		5				15	16	−4
19	Dr. L.			−5	10	72	5	−10	99
20	Nurse R.	20	7				12	6	34
21	P. W.	20	6				15	12	12
22	J. B. F.	48					Exp. 22·5 Insp. 18·5	12·5 15	30 12
23	Dr. G.	45		−2·5	10	59	Exp. 20 Insp. 17	6 8	36 47
	Do.			−4	12	64			
24	Sir D. F.	60	6	0	6	45	+ 2	+ 1	34
25	Mr. S. W.	55		−7·5	12·5	76	15	7·5	34
26	Dr. G. B.						10	4	40
27	G. E.	30	7·5	2	10	34	12·5	6	35
	Do.						15	6	41
28	Miss F.	25	9	2·5	8·5	29	12	7	28
29	Mr. G.						7	6	9
30	T. S. .... (Situs inversus)						10	12	−10
31	Captain E.						9	− 4	87
32	Dr. V.	56		2·5	10	31	Insp. 15 Exp. 17·5	7·5 5	34 48
33	G. R. M.	29		10	4	24	Insp. 12·5 Exp. 13·5	7·5 6	Insp. 27 Exp. 38
34	Dr. S. S.	45					12	1·5	58

No.		Age.	Trans-verse.	Right superior.	Left superior.	α superior.	Right lateral.	Left lateral.	α inferior.
						°			°
35	Miss E. B. G. R.		10	1	8	38	Insp. 8 Exp. 9	4·5 3·5	29 41
36	Miss M. F. H.		12	−4	12·5	63	9	− 4	70
37	Miss M. M. A.		15	−2	14	52	7	− 7	90
38	Miss M. G. B.		9	2	10	34	12	4	45
39	Miss N. L.		10	−2	10	60	9	2	52
40	Miss P. W.		5	4·5	7	33	8	6	16
41	Miss H. S.		3	2·5	6	22	10	6	27
42	Miss H. H.		7·5	0	8	45	7	4·5	23
43	Miss M. E. F.		7·5	5·5	0·5	40	9	4	38
44	G. W.	50	8				9	3	45
45	L. B.	35	6				Exp. 10 Insp. 9·5	6 7	27 17
46	Mrs. W.		7				Exp. 10 Insp. 9·5	5 6	34 24
47	Mr. A. A.		6				Exp. 8 Insp. 7·5	4 5	34 22
48	Sir T. L. B.		15	−2	14	53	7·5	− 6·5	88
49	M. M.			3	9	27	15	10	22
50	Prof. H.		10	2	10	34	13	5	42
51	Dr. M.	46	6	2	7·5	30	17·5	14	12
52	Dr. K.		6	−2	6	64	+ 5	+ 3	27
53	Dr. G. O.		12·5	−3	13	58	7	− 5	85
54	Prof. M.	54	6	−0·5	5	50	10	4	41
55	T. H. K.		6	2	7	29	15	10	22
56	Dr. W.		4	2·5	5	18	6	3	34
57	Miss N. T.	*c.* 20	5	−0·5	5·5	50	9	4	38
58	Miss G. H.	*c.* 20	7·5	1	9	39	Insp. 15 Exp. 16·5	8·5 7	29 39
59	Miss L.	*c.* 20	6	1	5	34	Insp. 10 Exp. 11	6·5 5	23 37
60	Dr. H.	*c.* 50	8				5·5	− 2·5	37

Acineta tuberosa: *A Study on the Action of Surface Tension in Determining the Distribution of Salts in Living Matter.*

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[Plates 14 and 15.]

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I. *Introduction: The General Effects of the Action of Surface Tension.*

The distribution of salts in living matter is supposed, in the current conception of the subject, to be on the whole the same as in ordinary fluids. Living matter is generally regarded as a semi-fluid, semi-viscid material in which the conditions, though not fully typical of those which obtain in a fluid like water, are, nevertheless, such as to allow the substances that are dissolved in it to be distributed uniformly throughout it. The only obstacle to this distribution may be presented by a membrane such, for example, as that which encloses or surrounds the cell nucleus. Elsewhere throughout the cytoplasm there is, it is believed, a free play of the force that determines the diffusion of the substance or substances dissolved, until uniformity in their dispersion obtains throughout the volume occupied by the cytoplasm.

This force is that postulated in the van 't Hoff theory of solutions extended to include the Arrhenius theory of dissociation. In this composite theory, as is well known, the material dissolved in a fluid is supposed to be in a state analogous to that of a gas, that is, in as rarefied a condition as if its molecules were isolated from each other and occupying alone the volume filled by the solution itself. The molecules of the solute and their ions, when they are dissociated, are thus supposed to be in translational motion, and the resulting pressure—the osmotic pressure—which they give, acts on the surfaces enclosing the fluid as the molecules of a typical gas act on the walls confining it. At every point in the system there would be, on this view, the same pressure and, in consequence, the number of molecules per given volume of the solution in any portion of it would be uniform.

On this conception of the force determining the distribution of salts in fluids there were based a number of views which have played a part in explaining physiological processes. Of these the most important is that which postulated that all diffusion, whether in the cytoplasm of a cell or through a living membrane, is due to osmotic pressure acting as a driving force, the ultimate result of whose action would be to equalise the pressure throughout the cytoplasm or on both sides of the membrane. This reduced the processes of secretion and excretion, as well as the diffusion into and from cells, to the operation of gas laws.

This explanation of the force and the conditions that make for the diffusion of solutes, not only in physical solutions but also in living matter, has obtained and still obtains a wide acceptance. The very simplicity of it, the support it derived from a considerable range of experimental evidence, and the unifying effect it appeared to exercise in a large number of phenomena manifested by solutions and gases, told very strongly in its favour, and eventually revolutionised the aspect from which all the problems of osmosis and diffusion were viewed.

Criticism, however, was not silenced. It was seen that there were phenomena which not only could not be explained in that way but also were irreconcilable with the explanation. These were physical and physiological. Of the physiological only is there concern here. In one of these, that observed in renal excretion, the concentration of the urine is much greater, ordinarily, than that of the blood plasma from which it is derived through the activity of the kidney tubules. In other words, the osmotic pressure of the product of renal action is greater than that of the blood. This cannot be explained by the van 't Hoff-Arrhenius theory, the only conceivable result of which would, in such a case, be approximately an equality of pressure or that the urine formed would not exceed in concentration, and, therefore, in osmotic pressure, the blood plasma itself.

The failure of the van 't Hoff-Arrhenius theory to explain this and other physiological results of a like character does not put the theory out of court in explaining many physical phenomena. It still may be regarded as of value in accounting for these, though even in this respect it may be looked upon as beset with limitations. In the physiological sphere its application is of much less service and, were it here the last word in the way of an explanation, the causation of a few physiological phenomena would ever remain an insoluble problem.

In recent years the aid of other factors in explanation of certain physiological phenomena has been sought for, and, as a result, attention has been specially directed to the principle of surface tension. The participation of this force, although considered as a factor in the causation of amœboid and contractile movement ever since 1869, was not suggested as influencing the distribution of salts in living matter till 1910, when the author advanced the view that surface tension plays an all-important *rôle* in determining the localisation of salts and other solutes in cells and tissues, and in controlling the diffusion through living membranes that brings about the formation of excretions and secretions.

It was the observations derived from the microchemical study of the distribution of potassium salts in living cells that led to this view. The compounds of this element are amongst the most soluble of all known salts. Only two of its salts are under certain conditions insoluble in water, and these are the triple salt, the hexanitrite of cobalt, sodium and potassium, and the double salt, the potassium platinum chloride. Neither of these is found in the natural world, and therefore the salts of potassium found in living tissue, when aggregated in masses or layers in a cell, cannot be so localised as a precipitate. Some other explanation for this localisation had to be sought for, and the author, after full consideration of all the facts involved, claimed that the localisation observed is a condensation due to the influence of surface tension.

How such a condensation may develop through surface tension may be recognised on an examination of the results of the action of the Gibbs–Thomson principle of surface concentration. This law or principle may be stated in a few words. It is to the effect that when a substance in solution increases the surface tension of a fluid system (*e.g.*, a drop of water) it is less concentrated in the surface layer than in the rest of the system, while a substance that lowers the surface tension of the system is more concentrated in the surface film than it is in the rest of the system. It has also been found by Lewis and others that solutes which raise the surface tension at a water–air interface as well as those which lower it, also lower it at a liquid–liquid interface and at a liquid–solid interface and undergo condensation there, as a result, it is understood, of the operation of the Gibbs-Thomson principle. At such interfaces the degree of condensation depends on the extent of the diminution of surface tension as well as on the concentration of the solute throughout the fluid system, but, assuming the application of the gas laws to dilute solutions, the concentration as deduced from Gibbs' formula for this value would be

$$S = -\frac{C}{i\mathrm{RT}} \cdot \frac{d\sigma}{dC},$$

where S is the surface excess per unit of surface area of the part affected, C the concentration of the solute throughout the fluid, σ the surface tension value, i R the gas constant, and T the absolute temperature.*

The value of S as experimentally ascertained was in a great many instances very small. Forch (3) found that in a normal solution of sodium chloride, which raises the surface tension of water, the deficit in the surface film was 0·024 mgrm. per square metre. Whatmough (16) with a normal solution of acetic acid, which lowers the surface tension of water, determined the surface excess to be 0·2 mgrm. per square metre, and this concentration increases by less than 15 per cent. even when the concentration of the acid throughout the system was increased eight-fold. Milner (13) estimated that in a sodium oleate solution of 0·00204 gramme-molecular strength the surface concentration of the sodium oleate was 0·4 mgrm. per square metre over that of the solution generally, but from the data furnished by Reinold and Rücker (15) regarding the conductivity of films made from a solution of 1 part of sodium oleate in 60 parts of water, Milner estimated the surface excess therein to be 2·4 mgrm. per square metre. The results of Benson (1) obtained with aqueous solutions of amyl alcohol of 0·0375 molar value gave a considerably higher value, the surface excess of amyl alcohol reaching a concentration of 0·0394 molar value, involving an increase of about 5 per cent.

Were these the only values to come into consideration, surface concentration, as a result of the action of surface tension, would be negligible, except for the solution of certain problems of very limited interest. There are, however, other experimentally determined values which make it plain that surface concentration is, under certain conditions, a very great factor in influencing the distribution of salts in solutions.

These values were recently determined by W. C. M. Lewis† (6, 7, 8) who, to ascertain them, employed ingeniously devised methods. The surfaces on which the condensations were studied were those of aqueous solutions in contact with hydrocarbon oil or with mercury. The oil or mercury was in the form of droplets or spherules of uniform size, the surface area of each of which was calculated from data derived from the total quantity of oil or mercury used and the total number of droplets or spherules formed. The hydrocarbon oil and the mercury were employed because they do not absorb or dissolve in themselves a trace of the solute from the solutions bathing the surface of the droplets or spherules.

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* For the development of this formula from the original values of Gibbs see S. R. Milner, *op. cit.*

† From the Muspratt Laboratory for Physical Chemistry of the University of Liverpool.

The hydrocarbon oil was employed in two ways: as an emulsion with the aqueous solution or as droplets, which, being lighter than the solution in which they were liberated, were allowed to ascend through a long vertical column of the solution. In the first instance a definite quantity of the oil was mixed with a known quantity of the solution and the mixture agitated for some hours in a shaking apparatus. The droplets of oil in the resulting emulsion were of approximately like diameter, which was in a large number of cases measured under the microscope. The average volume of each was determined, and from this and the total quantity of oil used the united surface areas of all the droplets present in the emulsion were calculated. The quantity of the solute in the emulsion, apart from that on the surfaces of the droplets, was ascertained by the application of the drop-pipette or stalagmometric method. As the concentration of the original solution was known, the difference between it and that ascertained stalagmometrically was the amount condensed on the united surface areas of all the droplets.

In the second method the hydrocarbon oil was allowed to ascend in droplets through a long column of the solution in a cylinder which terminated above in a broad cup-like receptacle. The apparatus was so arranged that the connection between cylinder and receptacle formed of rubber tubing could be cut off by a pressure clip. The oil droplets could freely ascend to the interior of the receptacle, but the flow of the contents of the latter back into the cylinder was reduced to a minimum. The quantity of oil used was known, and the total number of droplets which ascended through the column of liquid was determined from an average of counts made for several selected test periods. When the droplets had all ascended the connection with the cup-like vessel above was cut off by compression of the rubber tube below it, and the concentration of the fluid in the cylinder, whose volume was known, was determined. The difference between this concentration and that originally present, the total volume in the cylinder, and the united surface areas of all the droplets were the factors from which was deduced the concenttation of the solute on each cm.^{2} of surface area.

When mercury instead of hydrocarbon oil was used, the droplets, all uniform in size, fell through the fluid in a cylinder which ended below in a reservoir from which it could be cut off by the closure of a glass tap. As the volume of the mercury used was known and the number of droplets also ascertained through an average of the counts made for that purpose, the united areas of the surfaces of all the droplets were determined. The original concentration and volume of the solution being known and the final concentration ascertained, the amount of the solute condensed on each cm.^{2} of the surface area of the droplets was calculated.

Out of a large number of solutes used in these experiments only two, caffein and anilin, gave values which approximated the values postulated by the equation $S = -\frac{C}{iRT} \cdot \frac{d\sigma}{dC}$, while in the case of the others the experimentally ascertained values greatly exceeded the theoretic values. Thus, in a sodium glycocholate solution of 0·25-per-cent. concentration, the surface condensation on the droplets of hydrocarbon oil was 5×10^{-6} grm. per cm.^{2}, that is, the directly ascertained value was about 70 times the theoretic value. When mercury was used the ascertained value exceeded the theoretic about 25 times. In sodium oleate solutions the directly determined value was about 100 times the theoretic. With Congo red, methyl orange, and sodium hydrate the experimentally ascertained values were respectively 25, 43, and 20 times the values derived from the Gibbs equation. Even if errors in calculation were allowed for in every case, the excess of adsorbed solutes was still so great that Lewis suggested, as a possible explanation for the great discrepancy, that the adsorbed material is in a gelatinised or colloidal condition on the surfaces of the droplets of oil or mercury.

Lewis found also that in the case of inorganic salts the quantity adsorbed exceeded the theoretic amount, but it was chiefly the cation that was so affected. In silver nitrate the silver adsorbed was 5 times in excess; in cupric chloride the copper was 17; and in potassium chloride the potassium was more than 30 times the calculated amount. This excess of the cation is, in Lewis' opinion, probably due to electrical effects, since the oil used is negatively charged, and the potential difference between the oil and the water is approximately 0·05 volt.

The diameter of a water molecule is, according to Kundt and Warburg (5), $3{\cdot}39 \times 10^{-8}$, and, consequently, the diameter of its sphere of attraction at the oil–solution interface would be $6{\cdot}78 \times 10^{-8}$ cm., but Lewis, accepting the range as equivalent to that postulated by Parks (14), namely, $13{\cdot}4 \times 10^{-6}$ cm., and using also the value $5{\cdot}4 \times 10^{-6}$ grm. for the amount adsorbed per cm.^{2} from a 0·25-per-cent. sodium glycocholate solution as experimentally determined, calculated that the concentration of the bile salt in the superficial layer at the oil–solution interface was 40·3 per cent., or 160 times that obtaining in the rest of the solution. If, however, we assume that the range of molecular attraction is smaller than that postulated by Parks then the concentration of the bile salt at the interfacial surface must be extraordinarily high.

How such a concentration can obtain we do not know, but an explanation may be tentatively suggested. It is not certain that the solute adsorbed is confined to a deposit within the range of attraction of the molecules on the interfacial surface. It is not improbable that there is no sharp break between the concentration at the interfacial surface and that in the solution generally—that, in effect, there is a shading off between the two. The amount adsorbed does, indeed, depend on the surface tension of the solution at the interfacial line, and the effects of this surface tension do not extend beyond the range of molecular attraction of the molecules on the interfacial line, but in the case of the solutes which are adsorbed in such extraordinary excess as to suggest the formation of a colloidal or gelatinous deposit, the latter must tend to produce successively superposed interfacial deposits until equilibrium is attained, in which case the deposit may extend with lessening concentration through a distance from the interfacial surface equivalent, it may be, to several, if not many, times the range of molecular attraction.

These facts make it evident that in solutions in which interfacial surfaces, numerous as in emulsions and consequently of very great areal value, exist, the concentration of the solutes may fall very considerably through condensation of the solutes on the interfacial surfaces. This must result in lowering the osmotic pressure. The dissolved molecules free in the solution are fewer, and their pressure is less than that of the molecules in the simple homogeneous solution unaffected by surface tension, except at its boundaries or limiting surfaces. The osmotic pressure of a solution of potassium chloride contained in a beaker is, therefore, different in value from that of the same solution and like concentration in which numerous interfacial areas obtain through the presence of very numerous foreign non-soluble systems.

The elemental unit of living matter, the cell, is constituted of unhomogeneous material in which the essential constituents are chiefly in colloidal condition, that is, the constituents are minute particles or dispersoids, separating which is a fluid containing in solution the salts and other compounds characteristic of living matter. Such dispersoids present interfacial surfaces of very great areal value, and if the surface tension of the fluid at the fluid–dispersoid interface is lowered by the solute or solutes there must be condensation of them on these interfacial surfaces. This would lower very considerably the concentration of the solutions in the fluid diffused through the living matter, and thus the osmotic pressure, otherwise due to the presence of such salts in living cells, would be nearly correspondingly reduced.

Surface tension, therefore, through the operation of the Gibbs-Thomson principle determines in a very considerable degree the distribution of salts in the living cell. Further, between each cell of a tissue or organ and the lymph that bathes its surface there is an interface where the salts of the lymph may lower the surface tension, and in consequence undergo more or less of condensation there. This serves to lower the general concentration in the lymph, and the osmotic pressure is diminished accordingly.

That such surface and interfacial condensations of solutes in living tissues and organs can occur, and thus modify the distribution of their soluble constituents, though definitely indicated by the results of physical investigations, would largely remain of theoretical interest if there were not more direct evidence to this effect. Such direct evidence comes from investigation by microchemical methods of the distribution of salts in living cells. The microchemical localisation of salts in cells and tissues is as yet not advanced enough to enable us to determine the finer distribution in living structures of all its inorganic constituents, but along several lines it is complete enough to permit us to demonstrate a condensation of salts due to the action of the Gibbs-Thomson principle.

This localisation is most effective in the case of the potassium salts. Eight years ago the author found that the hexanitrite of cobalt and sodium represented by the formula $CoNa_3(NO_2)_6$, in an appropriately prepared solution, instantaneously precipitates potassium from its solutions as $Co(NO_2)_3,3(K/Na)NO_2+nH_2O$, in which the amount of the potassium ranges, according to K. Gilbert's (4) determinations, from 16·31 to 18·21 per cent. The sensitiveness of the reaction may be understood from the fact that, in a solution so dilute as 1 part of potassium in 275,000 parts of a solution formed chiefly of the reagent, the crystals of the triple salt, the hexanitrite of cobalt, sodium, and potassium, are formed.

In tissues the potassium is not always abundant enough to give with the reagent crystals of the triple salt, and in the vast majority of preparations the triple salt formed is evident only as a yellowish reaction, and, consequently, not sharply delimited in tissue preparations. If, however, the reagent is completely washed out of the preparation with ice-cold water, the application of ammonium sulphide, reacting as it does to form the black cobaltous sulphide, brings out, in an extraordinarily sensitive way, the distribution of the triple salt, and, therefore, of the potassium in it. This black reaction, as observed under the microscope, makes the distribution of the triple salt sufficiently sensitive to diagnose the presence of potassium when it is but 1 part in over 1,000,000 of tissue mass.

With this method of localising the distribution of potassium, the author succeeded in finding that, in a number of animal and vegetable cells, the potassium is condensed on surfaces in such a way as to make it very highly probable that surface tension is the only immediate factor involved in this condensation. Of these he has given an account in recently published communications (10, 11, 12).

More recently, however, the author has found a unicellular animal form, in which the action of surface tension in influencing the distribution of salts in it is, to all appearance, placed beyond doubt.

This organism is *Acineta tuberosa*, a Suctorian Protozoan of marine habitat, a form which permits readily of technical manipulation and microscopic examination, especially for microchemical purposes. Some, at least, of the salts of sea water penetrate its cytoplasm, and amongst these certainly are those of potassium, of which the chloride is apparently the most abundant. The distribution of this element in the cytoplasm of this organism was carefully investigated, and the results were found to be of such significance as to justify the detailed description of them given in the following pages.

II. *Methods of Investigation.*

The specimens of *Acineta tuberosa* used in these observations were found in abundance attached to brown filamentous algæ growing on wooden wharves and floats, at and just below the surface of the water, near the Marine Biological Station of the Biological Board of Canada, in the Bay of St. Andrews, New Brunswick, in July and August of 1911. It was easy at any time during that period to get an abundant supply of these specimens by collecting a mass of the algæ, which was carried to the laboratory in a quantity of the sea water of the immediate locality. In nearly all cases the material so collected was used within a few minutes—20 at the most—after it was collected.

For the determination of the distribution of potassium in the *Acinetæ*, a mass of the algæ was lifted with a forceps from the sea water, and allowed to drop into a quantity of the solution of the hexanitrite of cobalt and sodium. For the method of preparing this reagent, the reader is referred to an earlier article of the author's (9). At the end of five minutes the mass of filaments was removed from the reagent and placed in ice-cold distilled water, which was renewed every three to five minutes, until, at the end of half-an-hour, all the uncombined reagent was completely extracted, and the only demonstrable cobalt compound present was that in the form of the triple salt, the hexanitrite of cobalt, potassium and sodium. The filamentous mass was now placed in a quantity of a mixture constituted of equal parts of glycerine and fresh or recently prepared ammonium sulphide, which gave the preparation a black reaction, due to the formation of the black cobaltous sulphide compound.

In the transference from one fluid to another, goose-quill points, glass needles, or platinum points were used, in order to avoid contamination of the preparations with iron or other metallic salts, which would tend to give a bluish-black, black or brown reaction with the ammonium sulphide.

The preparations were now made ready for examination under the microscope. For this purpose minute portions of the material subjected to the treatment described were teased out on a slide in a drop of a mixture of five parts of glycerine and one of ammonium sulphide, a cover-glass was added, and, after all the glycerine-sulphide mixture not included under the cover-glass was carefully removed, the edges of the cover-slip were luted to the slide with benzol balsam to prevent evaporation and to facilitate examination under high magnification with the microscope. Preparations so made have been found after 16 months to have retained all their original value and distinctness.

For revealing the structural and other characters of the *Acinetæ* fresh material was placed in 10-per-cent. formalin solution, in which also it was kept. This material was treated with a saturated solution of scarlet red in 70-per-cent. alcohol to show the distribution of fat in these organisms. Further fresh material was treated with Zenker's fluid, Flemming's chrom-osmio-acetic mixture, and with saturated aqueous solutions of mercuric chloride. The material so prepared was used to reveal the minute structure of the organisms.

III. *The Results.*

The structure of an *Acineta* can be seen from an inspection of a formol-scarlet-red preparation, such as is illustrated in Plate 14, fig. 1. The delicate lorica (*a*) is transparent and surrounds the organism, except at three points. Two of these latter are where the hillocks of cytoplasm, bearing the tentacles, project beyond the contour outline. The third is less distinct and is found at a point on the anterior border, midway between the two hillocks. It is a minute pore, where terminates the canaliculus which connects the central cavity of the cell with the exterior. Into this central cavity, which ordinarily is very minute, grows the bud of the cytoplasm by which the organism is reproduced. Such a young form is shown in fig. 1. The minute pore, or aperture, and the canaliculus may be seen most clearly in preparations made to show the distribution of potassium.

The cytoplasm is crowded with spherules of a proteid character which are best revealed by the scarlet red stain. These, through the action of the hardening reagent, shrink more or less, and thus the spherules appear to lie in cavities which they incompletely fill. Dissolved in the spherules is a slight amount of lipoid material to which is due the faint stain given them by scarlet red. In some preparations the spherules appear to take with scarlet red a deeper stain. This is in large measure due to the fact that in the unstained condition they are impregnated with a reddish brown substance absorbed by the tentacles from the cytoplasm of brown Algae (fig. 8). With this brownish material there is associated lipoid material. Hence the deep brownish-red stain which these spherules have after treatment with scarlet red.

Here and there in the cytoplasm one can detect the presence of fat droplets, which, however, are very minute and few in number. The cytoplasm itself, apart from the spherules, is very finely granular. Especially is this the case in the hillocks from which the tentacles originate. In these hillocks there are no spherules or coarse granules of any kind, and even in the vicinity of, and immediately below, the hillocks, there are very few spherules and large granules.

The central cavity in which the germinal bud develops is ordinarily very small, but it is enlarged as the bud grows. The wall of the cavity and the surface of the bud are in close contact (fig. 1). The canaliculus which connects this cavity with the exterior is always patent, although in ordinary preparations it may be invisible.

The nucleus is placed below the germinal cavity and is invisible in the fresh preparation. When a germinal bud is developing the nucleus becomes irregular in shape, a lobate prolongation of it extends into the bud, and this lobate portion is separated by constriction at its narrow part and forms the nucleus of the cell of the developing bud. The process of division is thus amitotic.

The tentacles are 25 to 40, or even more in number, and they are usually disposed in a radiate direction in all planes from the convex surface of the hillocks. Their diameter is about $1{\cdot}2$–2μ, and their length, though varying from individual to individual, does not exceed, at the most, 35μ, but ordinarily is not more than 27μ. The outer end is capitate and its diameter is usually about $2{\cdot}7\mu$, that is about half as much again as the average thickness of the tentacles.

The shaft of each tentacle consists of an axial portion constituted of very finely granular cytoplasm enclosed by an external sheath or layer of homogeneous material which, because of its slightly greater transparency and refringency, appears readily distinguishable from the axial substance. The thickness of this sheath is about $0{\cdot}4\mu$ in the capitate region of the tentacle. The sheath is an extension of the ectosarc, for, at the base of the tentacle, it passes directly into the clear homogeneous limiting layer of the hillock, which layer, in other Protozoa, answers to what is known as the ectosarc.

The axial portion of the tentacles is constituted of the ordinary cytoplasm of the organism. It is almost homogeneous in composition, or hyaline in appearance. The material composing it does not appear to be derived from the cytoplasm of the hillock, but from that at a considerable distance below the hillock. This is clearly shown in fig. 2, in which are represented the channels in the cytoplasm, along which flows the more fluid and very finely granular material constituting the axial portions of the tentacles.

The currently accepted view as to the action of these tentacles in the absorption of food is that they are hollow and are provided at their ends with a cup-like sucking organ through which the food enters and is carried by suction action into the cytoplasm. The existence of a cup-like terminal for each tentacle I am unable to establish. There is nothing to suggest the occurrence of such a structure. The structure of the capitate point is that represented in fig. 7. As is not infrequently the case, the surface film of the point may be deeply impregnated with fatty substance which is sharply demonstrated in formol-scarlet-red preparations, but lipoid material is found in droplets irregularly at other points in the film along the course of the tentacles.

The tentacles are not always straight. They may exhibit a slight or a marked curve, but, as a rule, only one, or at most several, in a group, are so affected. When the tentacles are being retracted they become straight and the capitate end of each is reduced in diameter until the latter equals the transverse diameter of the tentacle itself, which does not decrease, however much the longitudinal diameter may diminish. The retraction affects all the tentacles in the two groups on a form equally, and it may proceed until, finally, they are but slight prominences on the external surface of the hillocks. It is, indeed, very rare to find them completely withdrawn into the hillocks.

From all the phenomena involved in the extension and retraction of these tentacles it is to be inferred that alterations in surface tension are involved. These alterations may affect different portions of the surface of the organism. The protrusion of the tentacles may be due to an increase of the tension of the general surface while the tension of the film at the points where the tentacles originate may remain as before, or the tension of the general surface may be constant while the tension of the film at the points where the tentacles develop may diminish. There is the possibility also that, while the tension of the general surface may increase, that of the film at the points of origin of the tentacles may decrease.

Whether, however, the tension of the surface film over the general surface of the organism is raised or not is a question on which there is no evidence, direct or indirect. It is, nevertheless, tacitly assumed that in other organisms, which exhibit at points on their surface extensions of the protoplasm in the form of pseudopodia or flagella, the negative answer to this question is the correct one. It may turn out eventually to be the right answer, but, on *a priori* grounds, it is not improbable that in an organism like an Amœba the distribution of energy may be so adjusted that the tension over the general surface may be raised. In a specimen of Amœba which has been moving continuously in one direction for more than four or five times its diameter such an elevation of the tension must be continuously occurring, as otherwise the film at the point where the pseudopodium develops would spread over the whole surface and movement would quickly cease.

However much uncertainty there is on this point there is none on the question of relatively low tension in the films of the tentacles. The surface tension in these may be the same or lower than it was before they were protruded, but when they develop the tension is in all cases less than that of the general surface as otherwise there would be no development of tentacles.

There should, in consequence, be in the surface films of these tentacles the condition which promotes surface condensation as the result of the action of the Gibbs-Thomson principle. The solutes which lower the surface tension of the tentacles should be found in greater concentrations in their surface films than elsewhere in the cytoplasm of these organisms.

This is the case with the potassium salts. When these organisms, in the very active condition, are treated, after the manner described above, with the hexanitrite reagent, followed by washing in ice-cold water and by application of ammonium sulphide, the distribution of potassium salts thus revealed is, in the vast majority of preparations, like that represented in fig. 3. In such the potassium is seen to be localised in the surface films of the tentacles, at the interface formed by the maternal and germinal cytoplasms and at the interface formed by the cytoplasm and each of the included spherules. Elsewhere in the cytoplasm the potassium salt is so minute in quantity as to be undemonstrable by the reagent employed for that purpose.

The occurrence of potassium salts at the interface formed by the maternal and germinal cytoplasms is in part at least due to surface condensation. The film covering the germinal cytoplasm must have a tension less than that of the film of the adjacent maternal cytoplasm, as otherwise the germinal bud would not develop in the central cavity of the organism. The condition at the interface would then be more or less like that at the interface formed by a drop of oil suspended in water, in which salts, *e.g.* those of potassium, are dissolved. In both cases there would be a condensation of potassium salts from the enclosing or surrounding medium on, but not in, the surface of the enclosed object or system.

Some portion, however, of the potassium salts found to occur at the interface between the maternal and germinal cytoplasms must be explained as involved in the process of excretion. In the canaliculus connecting the germinal cavity and the exterior potassium salts are frequently found (figs. 3, 4) and these may extend in the form of a plug or mass through the pore in the lorica. Sometimes the salts so excreted do not reach the exterior, for if the external end of the canaliculus is not in line with the pore in the lorica the salts pass to the right and left in the grooves above the cytoplasm formed by the two parallel folds of the lorica. The position of these folds and the presence of potassium salts in them is shown in fig. 9, which represents a specimen as seen when its anterior surface is turned towards the observer. Even when the canaliculus is flush with the pore in the lorica potassium salts may be found in the groove for some distance to the right and the left of the pore (fig. 3, *a*).

The occurrence of potassium salts at the interface formed by the cytoplasm and each of the included spherules can be demonstrated only in a few specimens of *Acineta* and even when the demonstration is clearest it is nevertheless of such a character as to escape observation unless it is specially examined with that end in view. That there should be such a surface condensation of potassium salts in the cytoplasm on the surface of the spherules would follow from the fact that the spherules must have a different surface tension from that of the cytoplasm. Why this condensation is not evident in every specimen of *Acineta* cannot be explained unless it be that the surface tension of the cytoplasm at the cytoplasm–spherule interfaces is not, in the majority of *Acinetæ*, diminished as it is in the cytoplasm immediately adjacent to the germinal bud or in the surface film of the tentacles, in which case there would be little condensation of potassium at the cytoplasm-spherule interfaces.

The condensation in the tentacles is sharply confined to their surface films. This is shown particularly in fig. 6, representing the terminal portions of two tentacles greatly magnified. The coarse granules there revealed represent the crystals of the hexanitrite of cobalt, sodium, and potassium, blackened by ammonium sulphide. The crystalline deposit appears to be very voluminous but this is due to the fact that potassium forms only about 16 per cent. of the crystals themselves. Were, however, the potassium much less abundant than this the precipitate would not be crystalline but one finely diffused throughout the surface film. Such a finely diffused precipitate is sometimes observable between the crystals in the surface film of the head of the tentacle.

The potassium salt is more abundant in the capitate portion of the tentacle than elsewhere in the latter.

Contraction of the tentacles is rarely observed in specimens very recently taken from their habitat, but when it is developing the potassium salt diffuses from the surface films of the tentacles into the axial portions, and in consequence in such, when treated to reveal the potassium, the tentacles are black throughout (fig. 4). In certain of such preparations the cytoplasm of each hillock and of the immediately underlying part has also a dark shade which indicates that the potassium salt, as a result of the retraction, has begun to diffuse from the tentacles downward into the cytoplasm. When the tentacles are completely retracted the potassium is then diffused throughout the hillocks into which they are withdrawn and also downward into the underlying cytoplasm. In some specimens even the hillocks may be inverted and then one finds their outline marked out by the deep black reaction they give. In fig. 5, which represents this occurrence, a more marked diffusion downward into the cytoplasm is shown by the dark shade which becomes less and less marked the further downwards this diffusion proceeds. As this diffusion develops the potassium salts tend to condense more on the surface of the spherules than was the case when the tentacles were still extended, especially on those spherules which are found in the anterior half of the organism. This may be explained as due to the greater concentration of potassium salts in the surrounding cytoplasm in this region.

Occasionally in specimens one observes crystalline bodies of unknown composition in the cytoplasm, on the surface of which potassium salts may be condensed. The condensation on their surfaces has been observed to be more pronounced when the tentacles are partially or wholly retracted (fig. 5).

The salt of potassium most abundant in these condensations is probably the chloride. This was shown by the silver reaction. When living *Acinetæ* were placed for 30 minutes in N/10 solution of silver nitrate, to which some nitric acid was added, and afterwards exposed to bright sunlight for 10 minutes, a brown-black deposit of the reduced silver chloride was found in them only in the superficial films of the tentacles and at the germ-bud–cytoplasm interface, where the potassium reaction was obtained. This would seem to indicate that the potassium is present as chloride only, but as the reaction for the SO_4 of sulphates was, unfortunately, not applied one must admit the possibility of some of the potassium being present as sulphate. If, further, sodium, calcium, and magnesium, as chlorides, are present in these organisms they must undergo condensation on the same surfaces and interfaces, though possibly to an extent different from that obtaining in the case of the potassium salts, and, consequently, some of the haloid chlorine demonstrated as present may be united with them.

IV. *General Observations.*

The foregoing observations make it evident that surface tension controls the distribution of potassium salts in the cytoplasm of *Acineta*, and that, whenever the surface tension at a point changes, there results a redistribution of the salts, which conforms to the altered conditions of surface tension. The quantity of potassium so affected appears to be very great as compared with the amount which is diffused throughout the cytoplasm. In fig. 3 the surface films of the tentacles, and the interfacial surfaces between the maternal and germinal cytoplasms, seem to hold by far the vast part of the potassium in the organism. What is the exact proportion so condensed, as compared with that in the cytoplasm generally, cannot be determined, but the very marked concentration in the surface films of the tentacles, and the almost entire absence of a reaction for potassium in the cytoplasm elsewhere, suggests that the degree of concentration greatly exceeds the value $S = -\frac{C}{iRT} \cdot \frac{d\sigma}{dC}$. As already pointed out, Lewis found that the condensation of potassium on the surface of the droplets of hydrocarbon oil in a M/20 (0·373 per cent.) solution of potassium chloride was 5×10^{-8} grm./cm.^{2}, or thirty times the value $1{\cdot}7 \times 10^{-9}$ grm./cm.^{2}, calculated from the equation. In a solution of the concentration M/20, the hexanitrite reagent would give a dense precipitate, and a precipitate is given* when the concentration of potassium is as dilute as 0·00039 per cent., or M/10000 KCl. It is obvious, then, that the concentration of potassium chloride in the cytoplasm is below this value, while the concentration of the condensation in the surface films of the tentacles must be much above it.

The thickness of the layer of condensation in the films is probably not as great as appears indicated in the preparations. The thickness depends in part only on the diameter of the molecules forming the surface films. If the molecules were those of water, they should have, according to Kundt and Warburg, a diameter of $3{\cdot}39 \times 10^{-8}$ cm., and a range of molecular attraction equal to $6{\cdot}78 \times 10^{-8}$ cm. If, further, the surface tension in the films were as low as that of the solution in contact with the oil droplets in Lewis' determinations, and the concentration in the cytoplasm were M/20, then the concentration in the condensation would be $(5 \times 10^{-8} \times 100) \div (6{\cdot}78 \times 10^{-8})$, or 73·7 per cent. of potassium; 73·7 per cent. of potassium is equivalent to 140·4 per cent. KCl, which is an impossibility. With a solution concentration less than M/20, on the other hand, the concentration of the condensation in the surface films would still be high, probably higher than if it corresponded to the value of the Gibbs equation for M/20, that is, $(1{\cdot}7 \times 10^{-9} \times 100) \div (6{\cdot}78 \times 10^{-8})$, or 2·5 per cent. of potassium, which is equivalent to 4·76 per cent. KCl, or 0·638 M.

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* The precipitate is found at the bottom of the test-tube containing the mixture of the solution and reagent, after it has been allowed to stand for some hours. The precipitate is also similarly given when the potassium chloride is of the concentration M/20000 in a mixture of the solution and reagent. Crystals of the precipitate may, however, be found in a drop of the mixture examined under the microscope a few minutes after it is made. (The mixture in these cases should consist almost wholly of the reagent.)

If the surface film were constituted of molecules of protein in which water was absorbed, the thickness of the zone would be very much greater than if it were formed of water alone, and this would provide for a concentration of potassium chloride much less than the impossible 140·4 per cent., yet greater, perhaps very much greater, than the lower limit, 4·76 per cent.

With such a concentration in each superficial film, the precipitate would be marked, but this would not be confined to the surface film, for the crystals formed would project into the underlying zone. This, perhaps, explains why the deposit seen under the high powers appears to have such a thickness as indicated in fig. 6.

What is the source of the potassium found in these condensations?

The amount of potassium found in the sea water around the wharf of the Biological Station at St. Andrews, as ascertained from analyses made by the author, ranges from 0·0272 per cent. to 0·0353 per cent. according to the tide. As chloride the potassium of the higher concentration would correspond to 0·0673 per cent. or slightly less than M/110. The presence of potassium salts in sea water suggests that the potassium salt diffuses into the organism from without, but the author found that when a mass of the filamentous Algæ to which a very large number of *Acinetæ* were attached, was placed for 24 hours in a large quantity of filtered sea water the majority of the *Acinetæ* examined contained very little potassium, although the tentacles were protruded. Further, in a few of the *Acinetæ* in every preparation there was little or no potassium present. It may also be noted that sea water with a concentration of 0·035 per cent. of potassium will not give with hexanitrite reagent a precipitate of the potassium salt which will at all compare in density with the precipitate of the same salt in the superficial films of the tentacles, and it is consequently improbable that the much higher concentration of potassium salt in the tentacles is derived by diffusion from the sea water.

The only other source of the potassium in the condensations in the tentacles is the potassium of the material absorbed as food through the tentacles. The organisms which are the prey of marine *Acinetæ* are chiefly vegetable and the cytoplasm of these is charged with potassium which, with their other constituents, is absorbed through the tentacles. Sometimes, indeed, such organisms heavily impregnated with potassium may be found attached to the tentacles of an *Acineta*.

The potassium absorbed seems to play no part in digestion or metabolism and though its condensation in the superficial films of the tentacles would indicate that it lowers their surface tension this diminution is not dependent wholly on its presence. The fresh-water form *Podophrya* (*Tocophrya*) *quadripartita*, which grows attached to *Cladophora*, *Vaucheria*, and other Confervoid forms, does not contain any potassium even when it is absorbing material from its prey. It does not become impregnated, even in the slightest degree, with potassium salts when it is placed in sea water for 24 hours. It is manifest, therefore, that the tentacles and the general surface of these organisms are ordinarily impermeable to the salts of their medium.

The low surface tension on the tentacles of *Acineta*, and at the points on the hillocks where the tentacles arise, is doubtless due to formation in these structures of a substance derived from the metabolism of the proteins and other constituents of the cytoplasm. When potassium salts are present they may co-operate with it in its action on the surface tension. What this substance is cannot definitely be indicated and conjecture is our only resource. On first consideration a lipoid is suggested. To determine whether such a body is present in sufficient quantity to permit it to be distinctly shown under the microscope, preparations of active *Acinetæ* hardened in 4-per-cent. formol were treated with a saturated solution of scarlet red in 70-per-cent. alcohol for 2 hours and after being washed for a few minutes in 60-per-cent. alcohol and then in distilled water, were mounted in 50-per-cent. glycerine. A careful examination of such preparations under the microscope revealed minute spherules of fat in the membranes of the tentacles. In some examples of *Acineta* these spherules were very numerous, in others they were few. Occasionally a distribution of fat like that shown in fig. 7 was observed. In such the superficial membrane of the terminal capitate end of the tentacle was deeply impregnated with fat or it presented the appearance of a mosaic formed of closely placed very minute spherules of fat. The superficial membrane, however, in the vast majority of the tentacles gave no indication of fat uniformly diffused throughout it.

Scarlet red is not a staining reagent to demonstrate the occurrence of soaps, or certain lecithins, which may be present in the membrane of each tentacle. The application, however, of a solution of osmic acid of 0·5-per-cent. strength to living examples of *Acineta* has failed to give any indication of the presence of these lipoids, while it demonstrated by a black reaction the minute spherules of fat revealed by the scarlet red.

There, consequently, appears to be little direct evidence that the low surface tension of the tentacles is due to the presence of fat. It may, indeed, be held that the presence of minute spherules of fat in their membranes predicates a saturation of the latter with fat, which, however, is so scanty that scarlet red and osmic acid fail to demonstrate it. If that were the case it would be difficult to explain why the saturation did not prevail over the whole surface of each hillock instead of being localised as it is.

The presence of minute spherules and other deposits of fat in the tentacles may be the result and not the cause of the low surface tension in the tentacles. Fats lower surface tension, it is true, but if the surface tension should, through the action of some non-lipoid substance, be diminished, the lipoid material, it is presumed, may condense where the tension is low and give appearances in the tentacles like those described.

The view that the tentacles of *Acineta* are hollow tubes open at their capitate ends finds no support in the observations of the author, and it follows that the food matter absorbed from the prey of these forms is not taken into the cytoplasm of the tentacles unchanged or absorbed indiscriminately by them. The homogeneous appearance of the axial portion of each tentacle while it is attached to the prey suggests that the material which is being taken into each tentacle is in a digested condition and that the digestion so effected occurs outside the tentacles or in the interior of the capitate ends. This involves the assumption that the tentacles secrete one or more ferments. Now, the existence of proteolytic ferments in the tentacles, even in minute quantities, would render the presence of amino-acids possible and these latter would bring about a diminution of surface tension which would maintain, if not originate, the extension of the tentacles. These ferments would be more abundant, in the state of hunger, and specially at the points where they would come most into service, and their activity, however slight, directed upon the proteins at points in the hillocks, would cause the protrusion of the tentacles there.

The presence of free amino-acids in living protoplasm is not unknown and especially in structures in which cellular activity is marked. In the growing points of vegetable structures free amino-acids have been found in quantities sufficient to render the demonstration of their presence certain. It is possible that in such growing structures they may play, amongst other parts, that one of lowering the surface tension at points on the cells where extensions of the latter occur. As there is not a great gulf fixed between the metabolism of an active vegetable cell and that of a vigorous animal cell the existence in the latter of free amino-acids on occasions is not improbable.

The observations just now advanced are, of course, largely of the order of speculation. They have been put forward because they are in a measure concerned with another problem for which the lipoid theory of membrane action has been proposed as a solution.

With a concentration of potassium in the superficial molecular layer of each tentacle giving a precipitate much denser than sea water gives with the hexanitrite reagent, the question arises why the potassium salt in the surface film of each tentacle does not diffuse into the sea water and equalise the concentration on both sides of the tentacle–sea-water interface. The presence of a lipoid in the superficial film would perhaps account for this inequality, for the lipoid would make the membrane more or less impermeable, not only to water but also to salts in the latter. This, however, does not aid much in the way of explanation, for if the superficial film owes its impermeability to a lipoid constituent there should be no penetration of the superficial film by a solution of a potassium salt from the cytoplasm underneath and no condensation of potassium would occur in the film. It is obvious, therefore, that the impermeability of the superficial layer of protoplasm of the tentacles cannot be due to a lipoid and that some other factor plays the important part. That force is surface tension, in all probability, for the force that condenses the potassium salt in the superficial film of each tentacle would hold it there, especially as the surface tension of the sea water is higher than that of cytoplasm and a diffusion of potassium salt into the sea water would tend to raise the tension of the latter, thus increasing, instead of decreasing, the inequality of the forces on both sides of the membrane–sea-water interface. If the surface tension of the external medium were equal to, or lower than, that of the superficial film of the cytoplasm of the tentacles, diffusion outward would take place in order to equalise the tensions on the two sides of the dividing interface. In support of this it may be pointed out that Czapek (2) has found that it is only when the external medium of vegetable cells has its surface tension lowered to 0·68 times that of pure water that the tannin diffuses from them into the external fluid.

Besides the influence that surface tension has upon the distribution of salts in the interior of living cells it has, it would appear, a very important effect upon the diffusion from them and, therefore, into them. The factors operating in cellular osmosis are, consequently, not so simple as those postulated in the van 't Hoff-Arrhenius theory of osmosis based on the results of the experiments of de Vries and Pfeffer. This compels a revision not only of the doctrine of the semi-permeable membrane as applied in physiology but also of not a few of the conclusions that were based on it.

V. *Summary of Results and General Observations.*

1. In *Acineta tuberosa*, a marine Suctorian Protozoan, the potassium salts are localised: (1) On the interface between the cytoplasm and each of the spherules strewn throughout the cytoplasm; (2) on the cytoplasm–germ-bud interface; (3) in the superficial film of each extended tentacle.

2. The quantity of potassium found at each cytoplasm–spherule interface is generally very minute, and may be observed only after careful examination in some preparations; the quantity on the cytoplasm–germ-bud interface is usually richer and more readily demonstrable; while the potassium is most abundant in the surface film of each extended tentacle.

3. The potassium in the remainder of the cytoplasm does not give a reaction with the reagent used, the hexanitrite of cobalt and sodium. With this reagent crystals of the triple salt, the hexanitrite of cobalt, sodium and potassium, may be formed in solutions as dilute as 1 of potassium in 275,000, but in microscopical preparations of cells appropriately treated with the reagent and subsequently with ammonium sulphide the sensitiveness of the reaction exceeds that limit, perhaps to the extent of demonstrating 1 part of potassium in 1,000,000. In any case the absence of a reaction in the cytoplasm generally is an indication that the potassium salt or salts present are in exceedingly attenuated dilution.

4. When the tentacles begin to retract, the potassium salt or salts in the film of each begins to diffuse into the cytoplasm of the main body of the organism. This diffusion results in a greater concentration at first in the cytoplasm near the base of the hillocks from which the tentacles take their origin, but, as the retraction proceeds, the diffusion progresses downward into the cytoplasm, which is now more or less deeply impregnated with potassium salts and the deposit on each cytoplasm–spherule interface becomes, as a rule, more distinct.

5. Complete retraction of the tentacles alone is rarely seen, but more often, though never frequently, one finds a complete retraction of hillocks into which the tentacles have been completely withdrawn. In the masses derived by retraction of such hillocks the potassium salt is still present, but a distinct reaction for potassium in the underlying cytoplasm shows that diffusion of potassium into the cytoplasm, as a result of the retraction, has proceeded.

6. The condensation of potassium in the superficial layer of the extended tentacles, and the diffusion downwards into the cytoplasm when the tentacles are being retracted, indicates that the Gibbs-Thomson principle of condensation, due to the action of surface tension, is the factor in bringing about the concentration of potassium salts in the superficial layer or film of each tentacle, and that the deposit on the cytoplasm–germ interface, as well as those on the cytoplasm–spherule interface, would appear to be due to the operation of the same principle.

7. Surface tension thus makes the concentration of potassium in the cytoplasm of the cell body of *Acineta* less than 1 in 275,000, and condenses it to an extraordinary degree in the surface film of each tentacle, and at other interfaces where the tension is low. Surface tension is, therefore, an all important factor in determining the distribution of potassium salts and, inferentially, of other solutes in active *Acinetæ*.

8. How the low surface tension is brought about which leads to the formation of the tentacles is not known. With microchemical methods for demonstrating fat, very minute spherules of fat are found in the superficial layer or film of each tentacle, and the superficial film of the capitate end of a tentacle may be, now and again, deeply impregnated with fat. Fat or lipoid substance may, consequently, be the cause of the low tension. It is, however, suggested that amino-acids are the substances which lower the surface tension.

9. The quantity of potassium salt condensed in the surface film of each tentacle appears to be of greater concentration than obtains in the sea water of the habitat of the organism. This inequality of concentration on the two sides of the surface-film–seawater interface is, it is explained, due to the action of surface tension in maintaining the condensation on that side of the interface where the surface tension is less. Lipoids, it would appear, are not concerned in preventing the exchange of potassium salts, for they should also prevent the penetration of the surface film by potassium salts derived from the underlying cytoplasm.

10. The current conceptions regarding cellular osmosis and the distribution of salts in living cells, based on the van 't Hoff-Arrhenius theories of solutions, must be revised.

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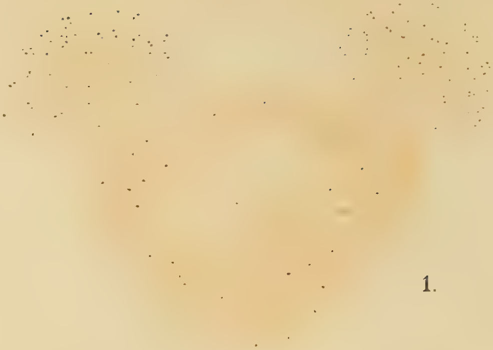

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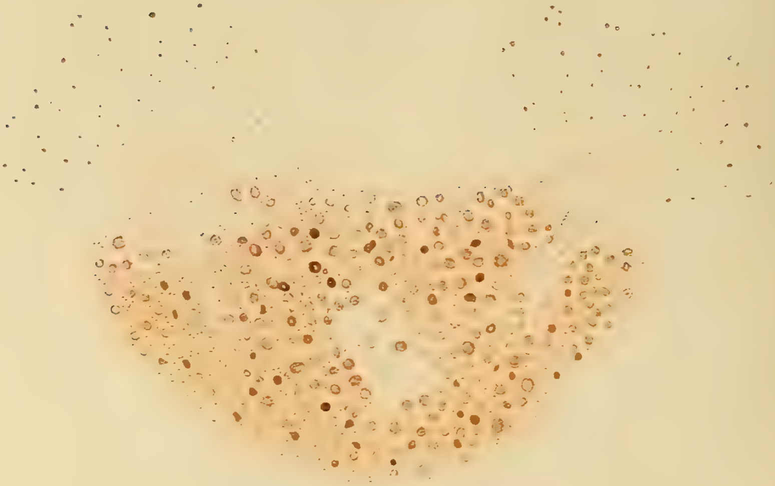

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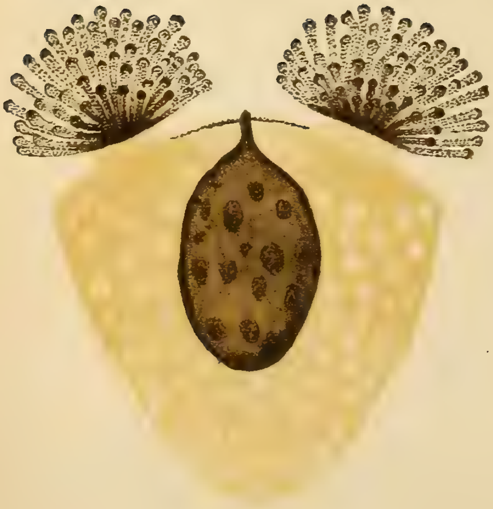

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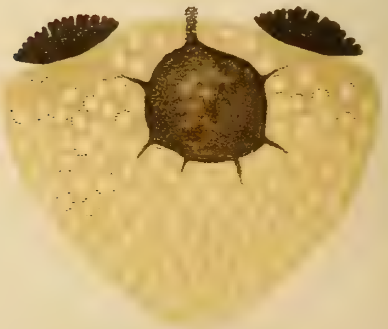

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VII. EXPLANATION OF PLATES.

Note.—The dark shading in the drawings indicates the cobaltous sulphide reaction developed from the treatment of the cobalt, sodium and potassium hexanitrite with ammonium sulphide. The dark shading thus represents the distribution of potassium throughout the organism.

Plate 14.

Fig. 1.—Mature specimen of *Acineta tuberosa*, treated to reveal the distribution of fat in its cytoplasm. Formol, scarlet red, glycerine. × 700.

Fig. 2.—Young adult specimen of *A. tuberosa*, showing the channels in the cytoplasm along which the axial contents of the extending tentacles flow. Formol, scarlet red, glycerine. × 1200.

Fig. 3.—Fully adult specimen of *A. tuberosa*, tentacles extended, treated to reveal the distribution of potassium in it. Cobalt sodium hexanitrite, glycerine-ammonium sulphide. × 750.

Fig. 4.—Fully adult *Acineta*, with tentacles partly retracted and the potassium salt or salts already to a certain extent diffused from the tentacles into the underlying cytoplasm. Cobalt sodium hexanitrite, glycerine-ammonium sulphide. × 750.

PLATE 15.

Fig. 5.—Adult specimen of *A. tuberosa*, with the tentacles and hillocks wholly retracted and the potassium salt or salts diffused in the underlying cytoplasm more than was the case in the form indicated in Fig. 4. Cobalt sodium hexanitrite, glycerine-ammonium sulphide. × 750.

Fig. 6.—The terminal portions, greatly magnified, of two of the tentacles of the specimen from which fig. 3 was drawn. Cobalt sodium hexanitrite, glycerine-ammonium sulphide. × 3200.

Fig. 7.—The terminal portions of two tentacles of a specimen of *A. tuberosa*, stained to show the distribution of fat in them. Formol, scarlet red, glycerine. × 1680.

Fig. 8.—Specimen of *A. tuberosa* unstained, to show the distribution in it of the pigment which it absorbs from the vegetable forms on which it preys. The hillocks and tentacles are free from it. Formol, glycerine. × 500.

Fig. 9.—Specimen of *A. tuberosa* seen with its anterior border tilted forward, showing the distribution of potassium salts in the grooves formed by the two parallel folds of the lorica. Cobalt sodium hexanitrite, glycerine-ammonium sulphide. × 450.

Carbohydrate Metabolism in its Relation to the Thyroid Gland.—The Effect of Thyroid Feeding on the Glycogen-content of the Liver and on the Nitrogen Distribution in the Urine.

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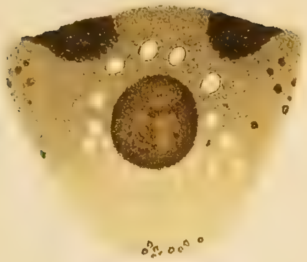
(Communicated by Sir E. A. Schäfer, F.R.S. Received June 10,—Read June 26, 1913.)

(From the Chemical Laboratory of the Physiology Department, Edinburgh University.

Introduction.

Our present knowledge of the relation of the thyroid gland to metabolism is based almost entirely on observations of the disturbances of metabolism produced by the diseases of the thyroid gland. By the application of physiological methods to patients suffering from Graves' disease or from myxœdema an increase in the total metabolism and in the nitrogen metabolism in Graves' disease on the one hand, a decrease in the total metabolism in myxœdema on the other, have been definitely established. From these facts the conclusion has been drawn that the secretion of the thyroid gland increases the oxidative processes, so that an inadequate functioning of the gland brings about the condition of obesity and depressed nitrogen metabolism, characteristic of myxœdema. As regards the carbohydrate metabolism it has been observed clinically that in Graves' disease there is sometimes a tendency to alimentary glycosuria; the opposite condition—an increased



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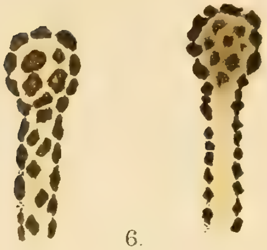

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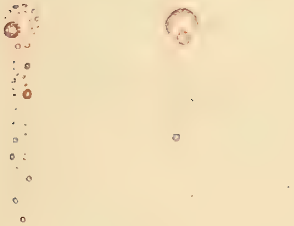

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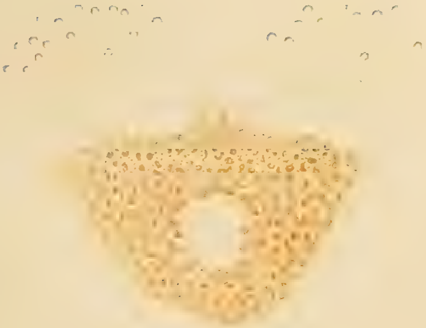

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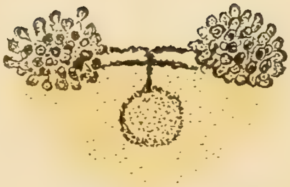

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tolerance for carbohydrates—has sometimes been found to occur in myxœdema.*

It is remarkable, however, that the attempts to verify these conclusions in the normal organism by producing experimentally a condition of excessive secretion of the gland—by means of feeding with thyroid gland,—or a condition of insufficient secretion of the gland—by extirpation of the thyroid gland—have on the whole not been successful. It is as yet not clear how the internal secretion of the thyroid gland produces its action on the metabolism. Nor do we know, at present, how the actions of this hormone on the different aspects of metabolism are related to one another.

Moreover different observers have obtained very contradictory results. Experimentally produced hypersecretion of the gland, brought about by feeding with thyroid gland, is frequently followed by a marked increase in the total metabolism and in the nitrogen metabolism, but in some experiments such an effect has failed to appear or is much less marked.† The conditions which determine the effect of thyroid administration on nitrogen metabolism are, as yet, not understood. And if the effect on the nitrogen metabolism does occur, the distribution of the urinary nitrogen is, as we pointed out in a previous communication,‡ the reverse of what one would expect to find on the general assumption that the thyroid secretion acts directly on the endogenous nitrogen metabolism.

A similar uncertainty exists with regard to the question how the carbohydrate metabolism is affected by the thyroid secretion. So far most experimental observations on this point have been made on thyroidectomised dogs, in which, owing to the close anatomical relationship between the thyroid and the parathyroid, the separate effects of thyroidectomy and of parathyroidectomy cannot easily be recognised. This probably accounts for the contradictory results obtained at first by different observers.

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* For a detailed account of the literature on metabolism in Graves' disease and in myxœdema the reader is referred to the articles by Rahel Hirsch and by von Bergmann, in Oppenheimer's 'Handbuch der Biochemie,' 1911, vol. 3 and vol. 4, to the article by Magnus-Levy, in von Noorden's 'Metabolism and Practical Medicine,' vol. 3, 1907, and to the third Lettsomian Lecture, by A. E. Garrod, 'Lancet,' 1912, p. 629.

† Marked effects were observed, amongst others, by:—In man: Bleibtreu and Wendelstadt ('Deutsche med. Wochenschrift,' 1895, p. 346), Magnus-Levy ('Zeitschr. f. klin. Medizin,' 1897, vol. 33, p. 269), Andersson and Bergman ('Skand. Archiv f. Physiologie,' 1898, vol. 8, p. 326). In dogs: Roos ('Zeitschr. f. phys. Chemie,' 1895, vol. 21, p. 19; *ibid.*, 1896, vol. 22, p. 58; *ibid.*, vol. 25, p. 12), Voit ('Zeitschr. f. Biologie,' 1897, vol. 35, p. 116), Oswald ('Zeitschr. f. phys. Chemie,' 1899, vol. 27, p. 39). No marked effects were observed by:—In man: Magnus-Levy, *loc. cit.*; in dogs: Underhill and Saiki ('Journ. Biol. Chem.,' 1908, vol. 5, p. 225), and a number of other workers, whose results are tabulated in the article by Rahel Hirsch quoted above.

‡ Krause and Cramer, 'Physiol. Soc. Proc.,' 1912, p. xxiii, 'Journ. Physiol.,' vol. 44.

Falkenberg,[*] Rahel Hirsch,[†] and Underhill and Saiki[‡] found the tolerance for carbohydrates to be diminished after thyroidectomy. In the experiments of Falkenberg and of Hirsch the term "thyroidectomy" is used rather loosely, and it is not clear whether the parathyroids were also removed or whether all, or any of them, were left behind, while in Underhill's experiments "both the thyroid and parathyroids were presumably completely removed." Eppinger, Falta, and Rüdinger,[§] on the other hand, failed to find such a condition after a thyroidectomy in which all four parathyroids were stated to have been left intact. The subsequent work of Eppinger, Falta, and Rüdinger[||] and of Underhill and Hilditch[¶] showed that complete removal of the parathyroids produces a transient but marked lowering of the tolerance for carbohydrates. The effects of partial parathyroidectomy differ according to the number of parathyroids removed. After the removal of one or two parathyroids the sugar-utilising power of the body is not decreased. Additional removal of the thyroid does not affect this result according to Underhill and Hilditch, who state that two parathyroids are sufficient to maintain normal control of the nutritional processes of the body. When three parathyroids are removed, the tolerance for carbohydrates is distinctly lowered, whether the thyroid is left intact or removed with the parathyroids.

The effects of thyroidectomy in the proper sense of the term are not so clearly understood as those of parathyroidectomy. This is partly due to the technical difficulty of removing the thyroid without at the same time removing or at least injuring the two internal parathyroids. Eppinger, Falta, and Rüdinger state, in their second paper, that thyroidectomy in the proper sense of the term, in which all four parathyroids are left intact, is followed by an increased tolerance for glucose. But we have been unable to find either in their first publication to which they refer, or in their subsequent paper, any record which would clearly demonstrate an increased tolerance after removal of the thyroid gland. McCurdy[**] observed an increased tolerance for carbohydrates in three dogs from which the thyroid together with two parathyroids had been removed. In one of these animals, however, the two remaining parathyroids were unintentionally injured to such an extent that the dog developed tetany and died. The fact that this animal too exhibited an increased tolerance for carbohydrates makes it difficult to give a correct interpretation to his observations on the effects of thyroidectomy.

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[*] Falkenberg, 10te Congress f. innere Medizin, Wiesbaden, 1891, quoted from Rahel Hirsch (see below).

[†] Rahel Hirsch, 'Zeitschr. f. experimentelle Pathologie u. Therapie,' 1908, vol. 5, p. 233.

[‡] Underhill and Saiki, 'Journ. Biol. Chem.,' 1908, vol. 5, p. 225.

[§] Eppinger, Falta, and Rüdinger, 'Zeitschr. f. klin. Medizin,' 1908, vol. 66, p. 1.

[||] *Ibid.*, 1909, vol. 67, p. 380.

[¶] Underhill and Hilditch, 'Amer. Journ. Physiol.,' 1909, vol. 25, p. 66.

[**] McCurdy, 'Journ. Exper. Med.,' 1909, vol. 11, p. 801.

Eppinger, Falta, and Rüdinger* further supported their arguments by observations on the behaviour of thyroidectomised animals to injections of adrenin. They stated that the glycosuria which takes place in normal animals after the injection of adrenin does not occur in thyroidectomised animals. But a repetition of these experiments by Underhill and his collaborators† failed to confirm these results. Underhill showed that one and the same normal animal under similar conditions differs considerably in its readiness to respond to the injection of adrenin by the excretion of sugar in the urine and that thyroidectomised animals respond to adrenin as readily as normal animals.

Whether excessive secretion of the thyroid hormone, produced by thyroid feeding, affects the carbohydrate metabolism of the normal organism has not been studied experimentally. Clinically it has been observed that thyroid administration sometimes produces a tendency to alimentary glycosuria.

From this brief review of the literature it will be evident that the relation of the thyroid gland to metabolism is not yet clearly understood. The effect of the thyroid hormone on the metabolism of the normal organism is uncertain and variable, so that a secure experimental basis is lacking and that considerable discrepancies exist between the observations of different authors. Most observers agree, however, in attributing to the thyroid secretion a direct stimulating influence on the nitrogen metabolism and on the total metabolism. To the latter factor they attribute, as a rule, the effect which the administration of thyroid gland has on the fat metabolism.

The Effect of Thyroid Feeding on the Glycogen-content of the Liver.

The observations recorded below show clearly that the thyroid hormone has a marked and very peculiar effect on the carbohydrate metabolism. If rats, kept on a carbohydrate rich diet (bread and milk) are fed for from two to eight days with relatively small doses of fresh thyroid gland (sheep's thyroid was used mostly) the glycogen-content of the liver falls so low that the glycogen cannot be estimated gravimetrically in the liver. One must, of course, take into account here, that in a rat only 4–6 grm. of liver tissue are available for analysis, so that a glycogen-content below 0·1–0·2 per cent. cannot be determined accurately. Such low values, which in a normal rat are found only after 10–15 hours' fasting, are described as a "trace" in the tables given below. In all our estimations Pflüger's method was used, the sugar after hydrolysis being determined by Bertrand's method. Unless otherwise stated the last dose of thyroid gland was given 24 hours before the animal was killed. In almost every case the liver of a thyroid-fed rat and that of a normal control rat were subjected to analysis at the same time. Both animals were kept on the same diet, both were fed at the same time, the amount of food taken being noted, both were killed simultaneously (by breaking the neck) a definite number of hours after a meal, the number of hours varying in different series of experiments. Such control estimations are particularly important in the case of the rats, since it has been shown* that in these animals the liver both forms and loses its glycogen more rapidly than the data given for larger animals would lead one to expect. All the animals examined took their food well and showed no signs of ill health.

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* Eppinger, Falta, and Rüdinger, *loc. cit.*

† Underhill and Hilditch, *loc. cit.*; Underhill, 'Amer. Journ. Physiol.,' 1911, vol. 27, p. 311.

The results obtained with rats, which are grouped together in Table I, were tested and confirmed by experiments on cats. These animals were also kept on a carbohydrate-rich diet (porridge and milk) and fed for one or two days repeatedly with fresh thyroid gland. Here, too, control estimations with the liver of normal cats were made in every case. Here, too, only a trace of liver glycogen was found in the thyroid-fed cats, except in one case, in which 0·37 per cent. of glycogen was found, a value considerably lower than those of the controls. By dissolving the total glycogen obtained from the whole liver in the other cases in a suitable small volume of water it was hoped to obtain sufficient material for a quantitative estimation, but even then the reduction was so slight that the amount of sugar present could not be determined. Examination of the urine contained in the bladder showed the absence of reducing sugar. The results of the observations on cats are given in Table II.

In a few experiments on rats the effect of a single administration of thyroid gland was studied (see Table III). There is then no very obvious effect on the liver glycogen, which is still present in definite amounts. There may be a diminution in the glycogen percentage of the liver and, in fact, in the few observations recorded in Table III the values found for the liver glycogen after one single administration of thyroid gland are below those of the control animals. But since the liver glycogen varies within wide limits, even in normal animals kept under similar conditions, a very extensive series of observations would be necessary in order to determine whether a single administration of thyroid gland is capable of affecting the liver glycogen. There is further the fact that individual differences exist in the activity of glands obtained from different animals even of the same species. This factor would be more likely to affect the results when the animals are fed only once, than when they are fed repeatedly with material from different animals.

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* Cramer and Lochhead, 'Roy. Soc. Proc.,' 1913, B, vol. 86, p. 302.

Table I.—Effect of Repeated Administration of Thyroid Gland on Rats.

Exp.	State of digestion.	Dose of thyroid and length of feeding.	Liver glycogen of thyroid-fed animals.	Liver glycogen of normal controls.
				per cent.
1	Bread and milk *ad lib.*	⅓ lobe every day for 5 days	trace	0·49
2	" "	" "	"	1·71
3	" "	" "	"	4·36
4	" "	¼ lobe every day for 8 days	"	—
5	3 hrs. after last meal ...	½ lobe every day for 5 days	"	1·7
6	" "	½ lobe every day for 4 days	"	4·2
7	" "	1 grm. dessic. gland on first day, 1 lobe fresh thyroid on second day	"	2·8
8	" "	1 lobe every day for 2 days	"	2·4
9	" "	" "	"	1·9
10	5 hrs. after last meal ...	1 gram dessic. thyroid on first day, 1 lobe fresh thyroid on second day	"	1·4
11	7 hrs. after last meal ...	" "	"	0·5
12	9 hrs. after last meal ...	" "	"	0·6

Table II.—Effect of Repeated Administration of Thyroid Gland on Cats.

Exp.	State of digestion.	Dose of thyroid and length of feeding.	Liver glycogen of thyroid-fed animals.	Liver glycogen of normal controls.
			per cent.	per cent.
1	3 hrs. after last meal	1 lobe twice a day for 1 day	trace	0·79
2	" "	1 lobe once a day for 2 days	"	3·03
3	" "	3 lobes three times a day for 1 day	"	3·88
4	12 hrs. after last meal...	" "	0·37	2·96
5	18 hrs. after last meal...	2 lobes on first day and 3 lobes the second day	trace	7·22

Table III.—Effect of Single Administration of Thyroid on Rats.

Expt.	State of digestion.	Dose of thyroid.	Liver glycogen of thyroid-fed rats.	Liver glycogen of normal controls.
			per cent.	per cent.
1	3 hrs. after last meal...	1 lobe 17 hrs. previously ...	1·1	4·2
2	6 hrs. after last meal...	" " ...	1·6	3·7
3	9 hrs. after last meal...	" " ...	0·5	1·8
4	11 hrs. after last meal...	" " ...	0·1	—

The Effect of Thyroid Feeding on the General Metabolism of Carbohydrates.

In the further study of this phenomenon we have been led by the following considerations. Two possibilities, which appear to be diametrically opposed to each other, suggest themselves as being capable of furnishing an explanation of this action of the thyroid hormone on the liver glycogen. Either thyroid feeding primarily increases the oxidation of carbohydrates in the organism or it primarily inhibits the function of the liver to form and store glycogen. In the former case one would expect to find the following combination of symptoms: (1) A formation of glycogen in the liver soon after a meal rich in carbohydrates followed by a disappearance of glycogen more rapidly than in the normal animals, (2) an increased tolerance for glucose, (3) a diminution of the blood-sugar, especially in the fasting animal. An inhibition of the liver function, on the other hand, would be reflected, (1) in the relative absence of liver glycogen even soon after a meal rich in carbohydrates, (2) in a diminished tolerance for glucose, (3) in a rise in the blood-sugar especially after a carbohydrate-rich meal.

Information on the behaviour of the liver glycogen in fed and fasting animals can be gathered from Table I. It will be seen that the effect of thyroid feeding on the glycogen-content of the liver is independent of feeding.

Experiments on the tolerance for glucose after thyroid feeding were made on dogs. A detailed account of these experiments will be published later; but the results may be briefly summarised here, as showing that thyroid feeding produces a slight but distinct lowering of the tolerance for glucose. A dog, for instance, which normally could assimilate 100 grm. glucose without any glycosuria supervening and only began to excrete sugar in the urine after the administration of 110 grm. glucose, had its limit of assimilation reduced to 90 grm. glucose after three days' feeding with fresh thyroid.

The behaviour of the blood-sugar is at present being investigated in this laboratory by Mr. R. J. M. Horne. These observations are not yet completed but they are sufficiently far advanced to show that there is at any rate no diminution in the sugar-content of the blood, but rather the reverse.

One must conclude, therefore, that the internal secretion of the thyroid gland, when administered to normal animals, has an inhibiting influence on the carbohydrate metabolism. But since the utilisation of carbohydrates by the organism is not markedly affected, as is shown by the comparatively slight lowering of the tolerance for glucose, it follows that the thyroid hormone acts specifically on only one aspect of carbohydrate metabolism in so far as it inhibits the formation and storage of glycogen in the liver.

The Effect of Thyroid Feeding on the Protein Metabolism.

This conclusion helps to explain a difficulty, already alluded to in the introduction, in the interpretation of the influence of the thyroid hormone on protein metabolism.

One distinguishes at present with Folin two forms of protein metabolism, the constant endogenous metabolism, which is independent of the protein taken in in the food, and the exogenous metabolism, which varies with the intake of protein in the food. Since thyroid feeding produces an increased protein metabolism even in the fasting organism, it follows that the thyroid hormone acts on the endogenous and not on the exogenous protein metabolism. One would expect, therefore, to find after thyroid feeding a marked increase in the excretion of uric acid and of creatinin, since both these substances are supposed to represent end-products of endogenous protein metabolism. We found, however, that the increased nitrogen excretion after thyroid feeding is accounted for almost entirely by the increased excretion of urea and ammonia, while the excretion of creatinin and of uric acid is either not increased at all or only very slightly.

This difficulty would appear to find its explanation in the fact that thyroid feeding affects carbohydrate metabolism in the manner described above. For the distribution of the urinary nitrogen after thyroid feeding is very similar to that which presents itself when carbohydrates are withheld from the diet. In the latter case, too, there is a marked increase in the nitrogen excretion,[*] even when no protein is given in the food, and here, too, the increased nitrogen output is due, almost entirely, to an increase in the excretion of urea and ammonia.[†] A further similarity is to be found in the appearance of creatin in the urine both after thyroid feeding[‡] and after withdrawal of carbohydrates[†] or in disturbances of carbohydrate metabolism such as diabetes mellitus[§] or phlorhizin diabetes.[§][||]

In order to demonstrate this similarity as clearly as possible, quantitative analyses of the nitrogenous urinary constituents have been carried out on one of us; (1) on a diet, creatin,- creatinin- and purin-free, but containing different amounts of carbohydrates, (2) on the same diets after thyroid feeding, (3) on a similar diet without carbohydrates.

The results are given in Table IV. Experiment 1 shows the effect of

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[*] Landergren, 'Skand. Archiv f. Physiologie,' 1903, vol. 14, p. 112; Kayser, quoted from Landergren.

[†] Cathcart, 'Journ. Physiol.,' 1909, vol. 39, p. 311.

[‡] Krause and Cramer, 'Phys. Soc. Proc.,' 1912, p. xxiii, 'Journ. Physiol.,' vol. 44.

[§] Krause and Cramer, *ibid.*, 1910, p. lxi, *ibid.*, vol. 40; Krause, 'Quart. Journ. Exp. Physiol.,' 1910, vol. 3, p. 289.

[||] Cathcart and Taylor, 'Journ. Physiol.,' 1910, vol. 41, p. 273.

Table IV.—Subject, R. A. K.; age, 26 years; weight, 65 kgrm.

Date.	Urine.	Total N.	Urea N.		Ammonia N.		Uric acid N.		Creatinin N.		Creatin N.		Diet notes.
							Experiment 1.						
	c.c.	grm.	grm.	p. c.	grm.	p. c.	grm.	p. c.	grm.	p. c.	grm.	p. c.	
26. 1	1100	11 ·56	8 ·98	77	0 ·72	6 ·1	0 ·12	1 ·0	0 ·49	4 ·8	0		Porridge, bread, butter, potatoes and milk.
*27. 1	1500	13 ·72	10 ·86	79	0 ·81	5 ·9	0 ·21	1 ·5	0 ·54	3 ·9	trace		
28. 1	1500	15 ·75	12 ·21	77	0 ·99	6 ·3	0 ·22	1 ·3	0 ·57	3 ·6	0 ·13	0 ·8	
							Experiment 2.						
20. 2	1370	3 ·37	2 ·02	59	0 ·27	8 ·0	0 ·17	5 ·2	0 ·52	15	0		Potatoes, fat, tapioca, butter and apples = 2900 calories.
†21. 2	1670	4 ·54	2 ·46	54	0 ·39	8 ·6	0 ·17	3 ·8	0 ·54	11	0		
22. 2	1950	4 ·37	2 ·38	54	0 ·40	9 ·0	0 ·17	3 ·9	0 ·51	11	0		
							Experiment 3.						
23. 12	1550	13 ·33	10 ·89	82	0 ·73	6 ·0	0 ·17	1 ·3	0 ·58	4 ·4	0 ·017	0 ·1	8 eggs, butter, cream, cheese, water, gelatine and potatoes.
24. 12	1250	14 ·49	12 ·55	86	0 ·95	6 ·5	0 ·15	1 ·0	0 ·48	3 ·3	0 ·028	0 ·2	The same diet but without potatoes.

* 10 lobes of thyroid given on 27. 1.

† 6 lobes of thyroid given on 21. 2.

10 lobes of thyroid gland = 16 ·2 grm. and contain about 1 ·8 mgrm. creatin nitrogen.

thyroid administration on a diet relatively rich in protein and poor in carbohydrates, while in Experiment 2 the diet was poor in protein and rich in carbohydrates. There is in both cases a marked rise in the nitrogen output due to an increased excretion of urea and ammonia, so that the percentage distribution of the urea- and ammonia-nitrogen remains practically the same. On the protein-rich diet there is also a distinct increase in the excretion of uric acid, and a slight increase in the excretion of creatinin, effects which are completely absent on the protein-poor diet. It is further interesting to note that no creatin is excreted when the thyroid is administered on a carbohydrate-rich diet.

Experiment 3 shows that withdrawal of carbohydrates from the diet is accompanied by a rise in the nitrogen-output which is due to an increased excretion of urea and ammonia. It is interesting to note that in spite of the fact that the subject of the experiment had been on a creatin- and creatinin-free diet for 24 hours before the beginning of the experiment, creatin was still being excreted and the creatinin excretion was above the normal for that person. There is a distinct increase in the excretion of creatin as the result of the withdrawal of carbohydrates from the diet.

A more detailed study of the effect of thyroid feeding on protein metabolism is at present being carried out by one of us. Here it is sufficient to point out that the similarity in the distribution of the urinary nitrogen in the two conditions (thyroid feeding and withdrawal of carbohydrates) suggests that the action of the thyroid hormone on protein metabolism is effected partly, at any rate, through its action on carbohydrate metabolism.

It would follow, too, that endogenous protein metabolism is many sided, more so than it has been supposed to be. There is that generally recognised form of endogenous protein metabolism, which is represented by the formation of creatinin and which is not very susceptible to the influence of the thyroid hormone. But there is yet another form of endogenous protein metabolism, quite independent of the former, which is under the influence of the thyroid gland and which would appear to have some specially close relation to the metabolism of carbohydrates.

General Discussion.

The most remarkable feature of the condition induced by thyroid feeding lies in the fact that the marked inhibition of the glycogenic function of the liver is not accompanied by a glycosuria or, at any rate, a very marked lowering of the tolerance for glucose, as the generally accepted view of carbohydrate metabolism would lead one to expect. This absence of any marked influence on the more obvious features of carbohydrate metabolism masks the relation which exists between the thyroid secretion and the carbohydrate metabolism, and has no doubt been the reason why this relation, although so frequently suspected, has not been demonstrated before. The fact that the glycogenic function of the liver may be so completely in abeyance, without producing any correspondingly marked effect on the tolerance for glucose, leads one to conclude that these two aspects of carbohydrate metabolism are independent of each other to a far greater extent than is generally supposed. Further investigations on this point are in progress.

The constancy with which the effect of the thyroid hormone on the liver glycogen is produced suggests this as a suitable test for the investigation of the relations which the thyroid is supposed to have to other internally secreting glands.

In conclusion it may be pointed out that the effects described in this paper are produced by the administration of thyroid gland to normal animals and represent therefore the conditions induced by a hypersecretion of the gland. It does not necessarily follow, although it is possible, that the thyroid hormone, in the amounts in which it is poured out into the blood in a normal animal, also has an inhibiting influence on carbohydrate metabolism. For it is a well known fact that physiologically active substances, which in large doses have a paralysing effect, produce a stimulating effect in smaller doses.

Summary.

When small amounts of fresh thyroid gland are administered for two or three days to rats or cats fed on a carbohydrate-rich diet, the liver will be found to contain only traces of glycogen.

This effect is due to an inhibition of the glycogenic function of the liver, not to an increased utilisation of carbohydrates. It is not accompanied by glycosuria, and other experiments on dogs, not recorded in this paper, show that the tolerance for glucose is only slightly diminished by thyroid feeding.

The action of the thyroid secretion on protein metabolism is effected partly through its action on carbohydrate metabolism, for the distribution of the nitrogenous constituents of the urine after thyroid feeding is very similar to that observed after withdrawal of carbohydrates from the diet or in disturbances of carbohydrate metabolism.

It is specially pointed out that the condition of the carbohydrate metabolism produced by thyroid feeding is unique. The bearing of these observations on current conceptions of protein and of carbohydrate metabolism is briefly discussed.

The expenses of this research were defrayed by grants from the Moray Fund.

Studies on the Processes Operative in Solutions (XXX) *and on Enzyme Action* (XX).—*The Nature of Enzymes and of their Action as Hydrolytic Agents.*

By E. Frankland Armstrong and H. E. Armstrong, F.R.S.

(Received June 13,—Read June 26, 1913.)

Our object in the present communication is to utilise the experience gained in the course of two convergent series of inquiries carried on during the past 12 years in the hope of arriving at a satisfactory solution of the problems of hydrolysis whether effected either by ordinary agents—acids or alkalies—or by enzymes.

The nature of the hydrolytic process has been discussed broadly in Part XXIV of the one series and the phenomena attending the dissolution of salts in water and the behaviour of saturated solutions towards precipitants has since been considered in Part XXV of the same series: much that has been said in these two communications will be of consequence in the present discussion. The views put forward with regard to the composition of water (S. VI)* and with reference to the part played by the class of substances which we have termed collectively *hormones*,† in altering the state of water and of substances dissolved in it, will also be found to have a bearing on the problems presented by enzymes. The present communication, it should be stated, is in the main an amplification of the views expressed in Part II of the Studies on Enzyme Action.‡

The interpretation of the phenomena we shall offer will also involve taking into account the views on residual affinity of the negative elements in particular and on the nature of the process of chemical change advocated by one of us in a communication brought under the notice of the Society in 1886 and in a Presidential Address to the Chemical Society in 1895.

The present communication, in fact, is the outcome of an inquiry, lasting over a long period, carried out with the object of arriving at a rational solution of some of the most fundamental of chemical problems—especially those relating to the interactions of water, interactions which it is now generally admitted are often of great complexity.

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* It will be convenient to refer to communications of the one or the other series as papers of the S. and E. series respectively.

† 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 588. *Cf.* 'Annals of Botany,' 1911, vol. 25, p. 507. As used originally by Bayliss and Starling, the term *hormone* had a restricted significance; we have applied it more generally to compounds which penetrate the differential septa of animal and vegetable structures.

‡ *Ibid.*, 1904, vol. 73, p. 500.

Definition of an Enzyme.—At the outset we are met by the difficulty of defining an enzyme. The state of opinion is well brought out in the opening lines of the recently published English edition of Euler's 'General Chemistry of the Enzymes':—

> "The name enzyme is given to animal or vegetable substances . . . . which are able to accelerate chemical reactions. The term enzyme is thus included in the much more general term catalyst. By catalyst we understand a substance which, without being required by the accelerated reaction or appearing among the final products, alters the velocity with which a chemical system strives to attain its final condition."

As one of us is responsible for the introduction of the word *catalyst,** we may be permitted to consider the significance of the term.

Catalysis.—It is noteworthy that the conception of catalysis, first enunciated by him in 1835, is discussed by Berzelius in his celebrated 'Jahresbericht' (vol. 15, p. 237), under the heading "Pflanzenchemie," in a section to which is attached the significant explanatory marginal note—"Some ideas on a hitherto unnoticed force active in living Nature in the formation of organic compounds."

At the outset, Berzelius refers to the difficulty of explaining the complex phenomena of organic life with the aid of the conceptions up to that time derived from the study of inorganic phenomena. He then draws attention to the discovery of a series of changes in which the agent appeared to take no permanent part in the change but was ultimately recovered unaltered in amount.

Thus he refers in succession to the formation of grape sugar from starch by means of dilute acids (Kirchof—1814): to Thénard's discovery of hydrogen peroxide, a substance which is readily resolved into oxygen and water: to Humphry Davy's observations on the effect heated platinum has in inducing the oxidation of the vapour of alcohol or ether: to Edmund Davy's discovery of platinum black, a substance which induces oxidation at ordinary temperatures: to the use that Doebereiner made of this discovery in constructing his well-known lamp: to Dulong and Thénard's observations on induced oxidation, showing that not only the platinum metals but also gold, silver and even glass could produce similar effects if sufficiently heated: finally, he refers to the discovery of diastase (Dunbrunfaut, 1830) and then discusses Mitscherlich's investigation of the formation of ether from sulphuric acid and alcohol and the manner in which this chemist had correlated with that of acids the action of diastase on starch.

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* 'Report of the British Association,' 1885, p. 953. We venture to deprecate the use of the expression "to catalyse"—both because it appears to us to lack euphony and to be unnecessary if not undesirable; for similar reasons, we regard catalyst as preferable to catalyser.

On account of the evidence afforded by his observations that water passes over together with ether, leaving the acid unchanged, when a mixture of sulphuric acid and alcohol is heated, Mitscherlich had supposed that the acid exercises the same power over alcohol that alkali exercises over hydrogen peroxide, arguing that its influence could not be ascribed to its affinity for water as the water was vaporised with the ether: he was further led to conclude that sulphuric acid and diastase act similarly on starch.

Hence Berzelius came to the conclusion that many substances, simple as well as compound, have the property in the solid and also in the dissolved state, of exercising an influence on compound substances which is quite different from that of ordinary chemical affinity, as they influence the occurrence of changes without their own constituents being necessarily concerned in the change—though there are cases in which this may happen. But he took care to point out that whilst he preferred to speak of the force contemplated as new it was presumably only the manifestation in a special way of the ordinary electrochemical properties inherent in matter. His views are summarised in the following statement:—

"Die katalytische Kraft scheint eigentlich darin zu bestehen, dass Körper durch ihre blosse Gegenwart und nicht durch ihre Verwandschaft die bei diesen Temperaturen schlummernden Verwandtschaften zu erwecken vermögen, so dass zufolge derselben in einem zusammengesetzten Körper die Elemente sich in solchen anderen Verhältnissen ordnen durch welche eine grössere electrochemische Neutralisierung hervorgebracht wird."

Berzelius finally calls attention to the possibility of "thousands of catalytic processes" being operative under vital conditions.

The meaning attached to the word catalysis in the interval has not only been vague but as often as not the term has been used to cloak ignorance and simulate understanding.

The following definition is given in a well-known dictionary: —

> "*Catalysis*—a decomposition and new combination supposed by Berzelius and other chemists to be produced among the proximate and elementary principles of one or more compounds by virtue of the mere presence of a substance or substances which do not of themselves enter into combination."

There is no doubt that gradually the term has been interpreted as implying an *action of presence*, the *catalyst* being regarded as a material which produces chemical change in another or other substances merely by contact, though it in no way has this significance etymologically. This idea has gradually supplanted that of a loosening down preparatory to and determining chemical change between substances in contact with the catalytic agent—the conception which appears to have been in the mind of Berzelius when he coined the term from the Greek *κατα* and *λυο.* The interaction of hydrogen and oxygen or of sulphur dioxide and oxygen in presence of platinum are cases in point: the "loosening down" of the molecules concerned is commonly overlooked and the combination effect alone thought of.

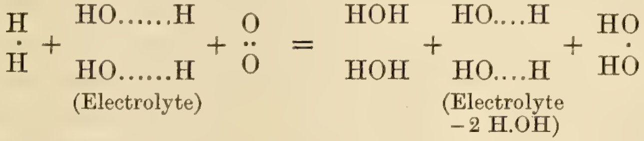
The definition which has been popular of late years is that quoted above from Euler but originally advanced by Ostwald. No proof whatever is forthcoming, however, that the catalyst merely alters and hastens the rate of a change already in progress. Whilst such an assumption is permissible, it is in no way necessary. A very large amount of evidence is on record showing that, in many cases in which it has been customary to speak of two substances as interacting, the change only takes place under special conditions in presence of a third substance of a particular type—in other words, that the catalyst determines the change: Brereton Baker's work, in particular, has afforded much proof of this kind but such evidence is entirely disregarded by the advocates of the view that the catalyst merely hastens change and it has nowhere received proper notice.

When chemical changes are regarded as electrolytic phenomena, as the several components of the system are all concerned in the change, it is obviously not an easy matter to decide which of the substances present is to be regarded as the catalyst. The interaction of hydrogen and oxygen, for example, is not determined by platinum alone but only takes place when an electrolyte is present to complete the circuit: the "loosening down" which Berzelius appears to have contemplated takes place immediately the circuit is formed and probably is consequent on the various attractions *reciprocally* exercised throughout the circuit. The platinum undoubtedly acts either by absorbing them at its surface or by combining with each of the two gases, thereby bringing them into circuit with the electrolyte.

On this account, the catalyst may well be defined as the agent which brings about the inclusion of the interacting substances in the circuit within which the change takes place so soon as the circuit is established, the electrolyte being the actual agent by which the change is effected.

Obviously, however, the electrolyte may, in some measure, be regarded as the catalyst and, as a matter of fact, it is generally so regarded in the case of the hydrolysis of ethereal compounds by acids.

The distinction between the view promulgated by Ostwald and that which we advocate lies in the fact that we do not admit that action is either possible or ever takes place between two non-electrolytes—such as hydrogen and oxygen, for example—but hold that action sets in only when a suitable electrolyte is present together with the two substances thought of as interacting directly, though in reality they interact only indirectly. Thus—



$$\begin{array}{c}\mathrm{H}\\ \cdot\\ \mathrm{H}\end{array} + \begin{array}{c}\mathrm{HO}\ldots\ldots\mathrm{H}\\ \\ \mathrm{HO}\ldots\ldots\mathrm{H}\\ \text{(Electrolyte)}\end{array} + \begin{array}{c}\mathrm{O}\\ \colon\\ \mathrm{O}\end{array} = \begin{array}{c}\mathrm{HOH}\\ \\ \mathrm{HOH}\end{array} + \begin{array}{c}\mathrm{HO}\ldots\mathrm{H}\\ \\ \mathrm{HO}\ldots\mathrm{H}\\ \text{(Electrolyte}\\ -2\ \mathrm{H.OH})\end{array} + \begin{array}{c}\mathrm{HO}\\ \cdot\\ \mathrm{HO}\end{array}$$

In such a case, the electrolyte is the catalyst, the occurrence of change being determined by and dependent on its presence: it does not merely accelerate the change but gives rise to it by making it possible. The change may be and is promoted, however, by the inclusion in the circuit of a substance such as platinum, which, by condensing or combining with the gases, promotes their association with the electrolyte. Therefore, if the term catalyst be restricted to materials which act merely by increasing the extent to which substances are brought into interaction and only as intermediaries, the definition given by the Ostwald school may be accepted as satisfactory: whereas, if the electrolyte be regarded as the effective catalyst, this is not the case, as the catalyst not only determines the occurrence of interaction but contributes, *ex hypothesi,* of its own substance to the change and is only recovered unchanged, *i.e.* undiminished in amount, because it is constantly being changed reversibly.

The Nature of Enzymes.—The enzymes are peculiar as catalysts not only because they are agents derived from natural organic sources which determine the resolution of a variety of compounds by hydrolysis but, more particularly, on account of their specific and limited activity: it is in this respect that they differ from most other catalysts. Each particular enzyme corresponds, if not to a single hydrolyte, at most to a series of compounds of one particular type. But until their specific nature be deciphered, it will be difficult to arrive at any final definition of enzymes.

The view that we have gradually been led to form of an enzyme involves the assumption that it has a double function—that of attracting or holding the hydrolyte and that of determining its hydrolysis: in other words, that the enzyme retains the hydrolyte in circuit while hydrolysis is being effected through the agency of an electrolyte itself formed from an active radicle present in the enzyme.

This twofold action we attribute to the presence in the enzyme of an *acceptor* together with an *agent.* According to this view, an enzyme is a composite agent in which the functions of a catalyst such as platinum black are combined with those of an acid catalyst.

It appears to us that the only interpretation that can be placed upon the facts as they are now known to us is that the acceptor is a radicle which is very closely allied to, if not identical with, a dominant group in the hydrolyte.

For example, we incline to the belief that the enzymes which cause the hydrolysis of the glucosides—the glucases—are themselves glucosides.

With regard to the agent, as the only hydrolytic agents known to us are either acids or alkalies and the latter act only on ethereal salts, not on etheric compounds such as the sugars, we are of opinion that, in all probability, the agent is an acid radicle so situated with reference to the acceptor that when the hydrolyte is attached to this latter it is in immediate or compatible proximity with it, a conducting path being formed between agent and acceptor by their association with the solvent and it may be also with a sufficient amount of some "salt" to render the intervening liquid an electrolyte.

In the case of enzymes which condition the hydrolysis of the carbohydrates and glucosides, the *agent* may well be the carboxyl radicle, CO.OH. It may be objected that the carboxylic acids are too weak—that the rate at which hydrolysis is effected by enzymes is far too great to be accounted for on the assumption that carboxyl is the effective catalyst. We shall discuss this point later on, merely remarking that we should not regard such an objection as a valid argument against the sufficiency of our postulate.

The efficiency of an enzyme depends, however, not only on the effective conjunction and simultaneous operation of the two elements we have termed acceptor and agent but also on its colloid character.

Manner in which Enzymes Act.—It is so generally held that the enzymes are colloids that we think it unnecessary to restate the arguments on which this conclusion rests but shall deal only with considerations derived from our own work.

In virtue of their colloid character, they are present in a liquid in suspension—their solubility being only apparent: results such as those obtained with urease, for example, cannot well be accounted for in any other way.

When hydrolysed by this enzyme, urea affords carbonic acid and ammonia. When the hydrolysis is effected in presence of an excess of either of these products, the rate is approximately a linear function of the time*: whereas, however, in presence of ammonia, the change is retarded, in presence of carbonic acid it is much accelerated.

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* E., XIX, p. 334.

A second point of importance to be noticed is the fact that the enzyme has maximum activity in solutions which are only moderately concentrated and that whilst dilution has but little effect, the rate of change becomes less and less as the concentration is increased.*

In no particular, therefore, is the change a "mass action effect," nor are the departures such that it can be supposed that the change is primarily "unimolecular" and subsequently varied owing to the occurrence of secondary changes: it is doubtful, also, if it be necessary to take the occurrence of reversible effects into account except perhaps in concentrated solutions.

[*Note added July* 30.—Bourquelot and Vardon's recent experiments† entirely justify this conclusion. These observers have digested aqueous solutions containing glucose and various proportions of methylic alcohol with emulsin and have determined the amount of glucose which remained unchanged when equilibrium was established. Their results are shown in fig. 1, in which the ordinates indicate the amount of glucose unconverted and the abscissæ the percentage by weight of methylic alcohol in the solutions.

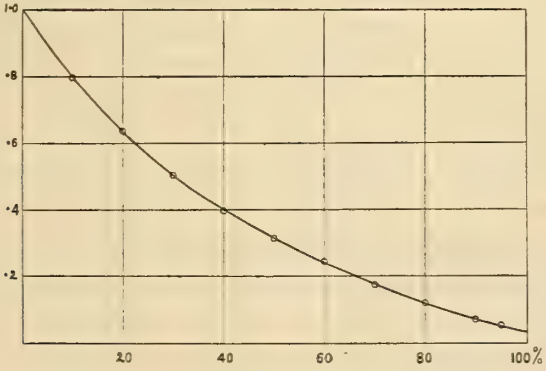

Fig. 1.

They have also shown that when solutions in 70-per-cent. methylic alcohol of varying amounts of glucose are digested with emulsin, the amount of glucoside formed increases proportionally to the glucose present up to about 12 per cent. of this latter but in more concentrated solutions at a diminishing rate: the proportion of combined glucose being 82·6 per cent.

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* E., XIX, p. 336.

† 'Compt. Rend.,' 1913, vol. 156, pp. 957 and 1638. *Cf.* 'Ann. Chim. Phys.,' [8], 1913, vol. 28, p. 145; 'Bull. Soc. Chim.,' 1913, No. 14, pp. i–xxviii.

in solutions containing up to 12 per cent. glucose and from 81·6 to 80·9 in solutions containing 16–30 per cent.

Moreover, the addition of β-methyl glucoside in advance serves to reduce the amount of glucose converted. Thus, when glucose alone was used (1 grm.), the amount converted was 0·826 grm. When an equal weight of glucoside was present, only 0·709 was converted and when 3 grm. of glucoside was used to 1 grm. of glucose, only 0·392 of the latter was etherified (*ibid.*, p. 1638).

It thus appears that the synthetic activity of the enzyme is affected in the same way as its analytic activity by changes in concentration. It is to be hoped that Bourquelot and Vardon will also determine whether the influence of the β-glucoside on the synthetic activity of the enzyme be in any way a preferential effect.]

But these are all conclusions at variance with the doctrine of the text-books. So far as we are able to judge, the analytic activity of enzymes is exercised more nearly in the manner first pointed out by Duclaux in 1898 and subsequently in 1902 by Adrian Brown and also by Horace Brown and Glendinning: *in each successive interval of time, the enzyme determines the hydrolysis of the same amount of the hydrolyte;* the observed departures from this rule may be attributed to the influence of the products of change.

The mental picture of the process we have been led to frame involves the following suppositions.

Firstly, that a colloid surface in water is necessarily a hydrolated surface, *i.e.* a surface to which molecules of hydrone—the fundamental molecule of water—are attached in such manner that their activity at the surface is greater than the average activity of the water in the neighbourhood. Secondly, that as a consequence of this property of the surface the hydrolyte is absorbed* from the solution, so that the colloid surface remains highly charged with the hydrolyte probably almost up to the point at which the supply in the solution is exhausted. Our assumption being that the enzyme is effective within a particular region, not over its whole surface, it is only necessary that the hydrolyte should be determined to this active region.

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* We venture to think that this term is sufficient for all purposes and that it is undesirable and unnecessary to introduce a special term (*ad*sorption) both because the uninstructed reader cannot attach any special meaning to this latter different from that conveyed by the familiar term and because the meaning which it is sought to give to it is not in reality different from that conveyed by the familiar term: no distinction is drawn by implying that a substance is sucked in *towards* a body *in* a solution rather than *from* a solution *by* a body, the process being one in which solution and surface are reciprocally concerned. The growing tendency to introduce special terms which the reader cannot understand unless specially instructed, whose meaning cannot be discovered easily, is to be deprecated on all grounds.

The partial or complete saturation of the surface of the colloid particles would serve, therefore, to promote the maintenance of a sufficiently constant supply of hydrolyte to this special region.

The hydrolyte attached to the acceptor, however, would not be permanently held but would oscillate between it and the liquid, so that only a certain proportion of effective contacts would be made—contacts during which the circuit would be completed wherein hydrolysis could and did take place. The rate of change would be determined by the rate at which these effective contacts occurred but would be relatively slow, in all probability.

After discussing the matter with Dr. Horace Brown, who has given special attention to such problems, we are inclined to think that the rate at which liquid diffusion takes place is probably so great that it is not necessary to take this into account as a limiting factor.

Influence of the Products of Change.—As the products of change accumulate in the solution, they affect the enzyme in various ways. It is to be supposed that the product immediately allied to the acceptor enters directly into competition with the hydrolyte and more and more takes its place as the amount present becomes greater. The carbohydrates and many glucosides are cases in point.

Products having no special configurational relationship to the acceptor section of the enzyme may act upon it in various other ways such as the following :—

(*a*) By neutralising it, as in the case of urease and doubtless also of pepsin and trypsin.

(*b*) By converting it into a derivative which is different in structure and no longer compatible with the enzyme—the action of some aldehydes and of quinone are cases in point.

(*c*) By changing the osmotic conditions in the solution and thereby altering the state of " hydrolation " at the enzymic surface of the acceptor and also of the agent. Probably any substance dissolved in the solution will act to some extent in this manner but such effects are specially noticeable in the case of " inert " materials such as the alcohols (hormones). The diminution in the rate of change which is noticeable when the concentration of the hydrolyte exceeds a certain maximum is to be accounted for, apparently, in this way. In explanation of this contention, we may point out that it is based on the assumption that in aqueous solutions all interactions take place at hydrolated surfaces—in other words, we regard both acceptor and hydrolyte as hydrolated and assume that they are brought into conjunction at their hydrolated surfaces.

Having thus called attention to the various factors concerned in the hydrolysis, we may now point out that our hypothesis involves the assumption that the relationship of the acceptor section of the enzyme to hydrolyte is not that of lock and key but that of a superposable and therefore practically identical radicle. As a matter of fact, the lock and key relationship, taken strictly, is unknown and even inconceivable: the only known relationship among similar substances is that of object and image: this is clearly not the relationship which holds between enzymes and their correlated hydrolytes.

We use the expression practically identical advisedly, in view of the fact that the tetramethylated methyl-β-glucosides* and certain glucosamine† derivatives are all hydrolysed by "emulsin" (prunase). Apparently therefore the spatial arrangement of the several radicles in the hydrolyte and the correlated acceptor must be the same in so far as the relative distribution of negative and positive is concerned but the negative groups admit of variation within certain limits—*i.e.* methoxyl may take the place of hydroxyl and apparently even the radicle NH_2 may to some extent be substituted for hydroxyl.

The enzymes which hydrolyse the glucosides generally may well be compounds of the glucoprotein class containing either α- or β-glucosidic radicles and capable therefore of hydrolysing either α- or β-glucosides, as the case may be, because their configuration harmonises either with that of the one or with that of the other type of compound.

To take another example, it appears not improbable that urease is an enzyme in which the urea residue in arginine is in suitable relationship with the carboxyl group.

Special Efficiency of Enzymes.—With regard to the efficiency of saccharoclastic‡ enzymes, two points have to be considered—the efficiency of the carboxylic radicle which we assume to be the agent and the special efficiency of a colloid mechanism such as we have pictured. It is well known that the efficiency of the carboxylic radicle varies greatly, being very low in the majority of acids but relatively high in formic acid and considerably higher in the substituted acetic acids, especially in trichloracetic acid; it is therefore conceivable that it may have a relatively high efficiency in the enzymes, especially if the region within which it is immediately active be one of high concentration.

As to the special efficiency of a colloid mechanism, as we have elsewhere pointed out, there is reason to suppose that when the sugars are hydrolysed by means of acids the proportion of acid effectively associated with the hydrolyte at any one moment is probably very small—as both are attracted by the solvent and therefore subject to constant separation at its call. Presumably, therefore, the efficiency of acids, though relatively very low, is actually very high (S. VII, XXIV).

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* Irvine and Cameron, 'Chem. Soc. Trans.,' 1905, vol. 87, p. 900.

† Irvine and Hynd, *ibid.*, 1913, vol. 103, p. 41.

‡ We regret that in earlier communications we have been guilty of using the indefensible terms "sucrose" and "sucroclastic" in place of "saccharose" and "saccharoclastic."

The colloid is little subject to such attraction and only the hydrolyte is specially attracted by the water; but owing to the fact that the colloid is present in an excessively finely divided state, the hydrolyte tends to accumulate at its surface and probably the attractive influence of the solution as a whole is largely overcome. A relatively large proportion of the hydrolyte is therefore brought into effective conjunction with the acid radicle; consequently this is placed under specially favourable conditions. The argument is applicable to enzymes generally whatever the nature of acceptor and agent.

Our hypothesis is one which renders it unnecessary to assume that enzymes obtained from a variety of sources which all function in a particular manner are one and the same substance; it is probable that the same acceptor and agent may be differently attached so long as they are appropriately placed to act in conjunction. It is conceivable, in fact, that a variety of enzymes may exist which are all capable of hydrolysing only one particular compound or type of compound but differ in activity. If, as appears to be the case, a given enzyme will act on compounds so different as say β-methyl glucoside and the corresponding β-methyl glucosamine derivative, it is clear also that a series of equivalent acceptors may give rise to corresponding enzymes which would all function similarly though probably with different degrees of readiness.

Our point of view is also one which admits of the existence of several classes of enzymes: for example, of enzymes which are compatible with the whole of the molecule they attack—it is not improbable that invertase belongs to this class—as well as of enzymes in which the acceptor is a group compatible with the one or the other section of the glucoside or other compound which it can hydrolyse.

Specific Character of the Enzymes.—The rigidly selective activity of the enzymes is in itself sufficient proof of their essentially specific character.

The fact that enzymes are known, such as α- and β-glucase (derived from yeast and the almond fruit respectively), each capable of hydrolysing a series of glucosides, is in no way subversive of this argument; it is easily accounted for by the assumption that each such enzyme carries an acceptor compatible with a group common to all the members of the series of glucosides and is, indeed, a corollary of the hypothesis.

Our conception of an enzyme is embodied in the two diagrams, figs. 2

and 3, representing the two amino-glucosides formed by the interaction of α- and β-glucose with the amino-radicle in a molecule of a complex albuminoid material.*

The models used serve to show the spatial arrangement of the atoms in the acceptor: actually, the atoms in the glucoside would be close packed and in immediate proximity to the main mass of the colloid represented in the diagram by a sponge and probably would constitute but a slight excrescence on its surface.

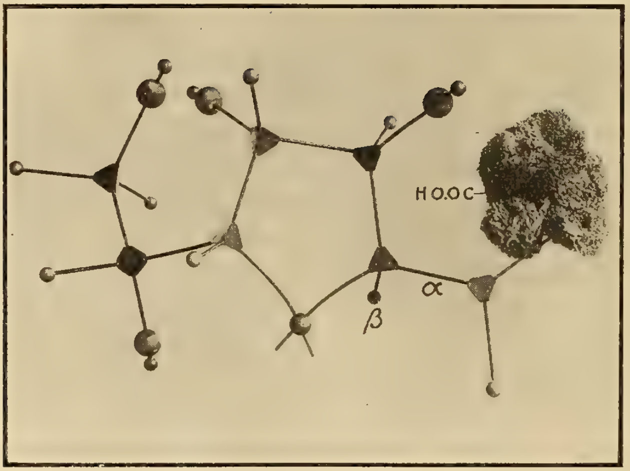

Fig. 2.—α-glucase.

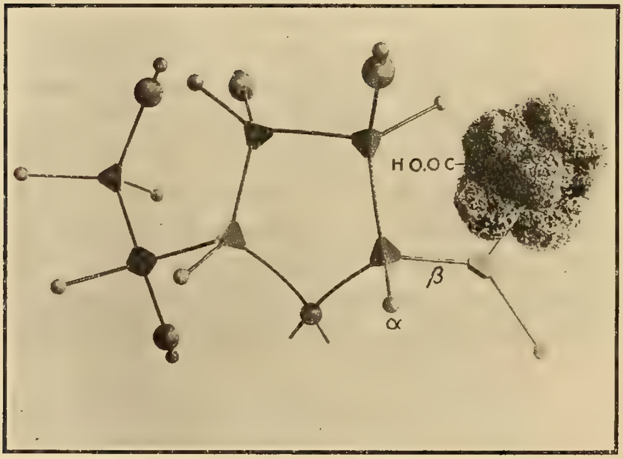

Fig. 3.—β-glucase.

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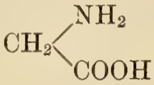
* *Cp.* van Laer, "Sur la Nature de l'Amylase," 'Bull. Acad. Roy. Belg.,' 1913, p. 395.

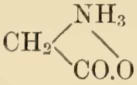
If both the carboxylic radicle and the amino-radicle to which the carbohydrate radicle is coupled formed part of some one amino-acid residue in the colloid complex, they would be in close conjunction and therefore self-protective; the "resting" enzyme may be thought of, in fact, not as an acid proper but as an internal salt of the glycine type:—

Aminoacetic acid



$\mathrm{CH_2}{<}\begin{matrix}\mathrm{NH_2}\\ \mathrm{COOH}\end{matrix}$

Glycine

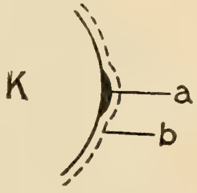


$\mathrm{CH_2}{<}\begin{matrix}\mathrm{NH_3}\\ \mathrm{CO.O}\end{matrix}{\diagdown}$

To unlock and render the "zymogen" active, it would be necessary to add a substance of superior acidic power—just as in the case of an indicator, which "indicates" only when either an acid or an alkali is added which is of superior strength.

It will be obvious that if brought into the proper apposition the molecule of an α-glucoside would fit the α-enzyme and the molecule of a β-glucoside the β-enzyme shown above and would fit it in such manner, moreover, that not only would contact be secured over the carbohydrate surface but the junction at which hydrolysis takes place in the glucoside would be in close proximity to the carboxylic radicle in the enzyme: it cannot be doubted that if an electrolyte intervened, hydrolysis would at once take place in such a system. The manner in which the electrolyte operates in such cases has been discussed in S. XXIV, p. 617, § 26.

In amplification of the argument advanced on p. 571, it may be pointed out that if each enzyme particle, in virtue of its colloid character, tend to absorb the hydrolyte so that the solution at its surface is relatively concentrated, the concentrated layer (*b*) would necessarily extend across the active enzymic area (*a*), as may be illustrated thus:—



The mechanism postulated is therefore such that the concentration of the hydrolyte would be raised and preserved in the neighbourhood of the active enzymic centre.

Action of Acids and Alkalies.—If we seek to interpret the effects produced by enzymes in the light of the hypothesis now advocated, it is obvious that one of the main conditions to be fulfilled is the maintenance of the freedom of the acidic (or alkylic) radicle which is the active hydrolytic agent.

It is probable that the amount of actual enzyme present in the preparations which are ordinarily used is so minute that the quantity of acid (or alkali) which would render it active initially, assuming that it is present either as an internal or as an ordinary salt, must be very small: therefore, if any further amount be required and the presence of acid (or alkali) in excess serve to accelerate the action of the enzyme, it is to be supposed that the acid (or alkali) neutralises some product or products of the change. This has been shown to be true in the case of urease, as ammonia retards the hydrolysis of urea by the enzyme whilst weak acids accelerate the change.

Assuming that it is a derivative of arginine, the enzyme urease may be represented by the following diagram, the section which the urea molecule would fit being that within the figure :—

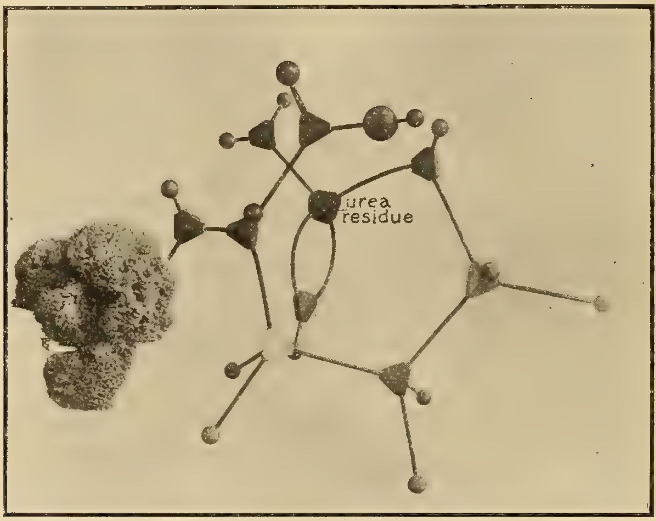


Fig. 4.—Urease.

The carboxyl group in such a compound would tend to unite with one of the contiguous basic NH groups, thus forming an internal salt. It would remain free if sufficient acid were present to hinder the formation of the salt but neutralisation would set in as ammonia was liberated from the urea : the enzyme would not be entirely thrown out of action, however, as the neutralisation would be more or less incomplete, as the ammonium salt would be dissociated by the water : its activity would be in great measure if not entirely preserved if an acid were present which neutralised the ammonia, so long as the effective acidity of the solution did not exceed a certain maximum—beyond which the acid would itself interfere by combining with the enzyme and perhaps also with the hydrolyte.

Apparently a not inconsiderable degree of acidity is essential in the case of peptic digestion. It is therefore not improbable that the active radicle in the enzyme is stronger than carboxyl and that it may even be a phosphoric residue—either PO(OH) or $PO(OH)_2$. As the products of change are more basic than the primary hydrolyte, in such a case the presence of acid would be necessary to maintain the freedom of the enzyme.

A similar argument may be applied to tryptic digestion in presence of alkali. The fact that the action takes place in presence of alkali cannot well be explained, in terms of our hypothesis, except by the assumption that the active agent of change is a basic radicle—perhaps an "ammonium" hydroxide. As the products of change—the glycines—are capable of neutralising bases as well as acids, the presence of alkali would serve to maintain the freedom of the basic radicle in the enzyme.

The saccharoclastic enzymes, as a class, appear to be active only in almost neutral solutions, a very slight degree of acidity being the most favourable condition. Faint acidity rather than alkalinity appears to be the natural condition of most living structures in which enzymes are present. But, as a rule, under natural conditions, enzymes are not subject to control by the products of their own action, as these are removed by diffusion more or less rapidly and cannot accumulate as they do in laboratory experiments.

Assuming that the enzymes are formed from internal salts (zymogens) by neutralisation, it is to be supposed that the degree of acidity or alkalinity which would be most favourable initially will vary according to the "strength" of the salt to be neutralised; at subsequent stages, variations may arise owing to the formation of products of varying "strength."

It has long been recognised that quantity alone is not the only factor to be taken into account in using acids but that these vary greatly in "strength." Thus, if cane sugar be subjected under strictly similar conditions to the action of quantities of chlorhydric and acetic acids which neutralise the same amount of alkali, the amount hydrolysed in a given time by the latter will not be 1 per cent. of that hydrolysed by the former. It is therefore not sufficient to determine the mere amount of "acid" present in a liquid by determining the amount of standardised alkali required to neutralise it—the *apparent acidity* of the solution: but the *effective acidity* must be ascertained.

Two methods of determining the effective acidity or strength of dilute solutions of acids are in use—the one is electrical and involves the determination of electromotive force, the other colorimetric; but the two have been applied in such a way that they are interdependent. A custom is growing up, which is much to be deprecated, of stating the results in terms of hydrogen-ion-concentration. Undoubtedly this method is not only one which can be understood by those alone who are instructed in terms of a special hypothesis—that of ionic dissociation—and have the necessary mathematical knowledge to grasp its significance but it is misleading in more than one respect. It has been contended, indeed, throughout this series of studies that the doctrine itself is purely hypothetical and not in accordance with facts generally.

The upholders of the dissociation hypothesis assume that the characteristic activity of acids is due to the constituent all acids have in common, the hydrogen ion (H) and that of alkalies to the hydroxyl ion (OH). Everything goes to show, however, that acids act as acids—that is to say that the negative or acid ion is as much concerned as is the positive or hydrogen ion; in fact, that the characteristic properties of an acid are in the main due to the negative radicle, the hydrogen radicle being no more characteristic of an acid than it is of an alkali.

Ex hypothesi, the passage of an electrical current through a solution takes place only through the agency of the dissociated ions. The slight conductivity of highly purified water is therefore attributed to the presence of a small proportion of free hydrogen and hydroxyl ions. The results are so interpreted that about 1 molecule in every 10,000,000 ($1 \cdot 05 \times 10^{-7}$ at 25°) is supposed to be in this condition; further, that if produced in a larger proportion than this under any conditions, hydrogen and hydroxyl ions at once unite to form neutral water. If a solution be acid, it is therefore supposed that hydrogen ions are present in excess of the proportion in which they are contained in water: if it be alkaline, the assumption is made that they are present in a smaller proportion.

It is difficult enough for non-mathematical readers to appreciate values stated in terms of the expression $x \times 10^{-7}$ or 10^{-y} but it is still more difficult for them to follow the method adopted by Sörensen,* the first to introduce order and one of the chief workers in this field, who uses the indices alone (the y values) as the *exponents* of the hydrogen-ion-concentration, so that values below 7 indicate alkalinity and those above 7 acidity. Such a system, moreover, has the disadvantage that when curves are plotted to indicate the relation between the effective acidity (or alkalinity) of the solution and enzymic activity, as logarithmic values are used instead of actual values, an altogether false and misleading shape is given to the graph.

Recognising the unsuitability of the method followed by Sörensen and others, James Walker† has recently advocated that acidity and alkalinity be referred to water as a standard. He puts the acidity and likewise the alkalinity of pure water as equal to 1: hence the product of the acidity and alkalinity of any solution is always unity. He proposes the use of a series of standardised solutions of the two phosphates KH_2PO_4 (acid) and Na_2HPO_4 (alkaline) in certain proportions. The relative acidity and alkalinity of such mixtures being known, it is easy to determine that of any given solution by matching the tint which it produces when mixed with azolitmin with that produced by one of the mixtures. The values given by N/15 solutions vary between a relative acidity of 300 and a relatively alkalinity of 20. Inasmuch as most enzymes show maximum activity within this range, such solutions afford very convenient standards. As illustrating the delicacy of the control, it may be added that whereas the relative acidity of a solution of N/15 acid phosphate is 300, that of N/10 acetic acid is 13,000.

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* S. P. L. Sörensen, "Études enzymatiques. II.—Sur la mesure et l'importance de la concentration des ions hydrogène dans les réactions enzymatiques," 'Comptes rendus des Travaux du Laboratoire de Carlsberg.' 8me volume. 1re Livraison, 1909.

† 'Journ. Soc. Chem. Ind.,' 1912, p. 1013.

In the past we have emphasised the need of carefully excluding all alkaline impurity in studying the action of saccharoclastic enzymes and have shown that the addition either of faintly acid or of amphoteric substances such as glycine was of material advantage in the case of invertase. Other workers have since used an acid phosphate for the same reason.

The method we have adopted in the experiments now to be referred to has been to extract dried yeast powder with the phosphate solution and after filtration to add a certain portion—about 20 c.c.—of the extract to a solution of α-methyl glucoside in 80 c.c. of the same phosphate mixture. The acidity of such a mixture is approximately, though not strictly, that of the original phosphate. On account of the yellow colour of the solution, it is impossible to determine the acidity exactly by the colorimetric method practised by Sörensen nor were we concerned to achieve such a degree of accuracy: for our purpose, it was sufficient to know that the solutions used varied in acidity.

As might be expected, an extract of dried yeast contains sufficient soluble material to provide a highly favourable medium. Thus, a solution made with ordinary distilled water containing carbon dioxide had a relative activity of 83 towards α-methyl glucoside; when distilled water free from dissolved carbon dioxide was used, the slightly lower value 75 was obtained; when a mixture of acid and alkaline phosphate in the proportions necessary to give neutrality was used the relative activity of the extract was 84.

The great variation in the results obtained with solutions of various degrees of acidity and alkalinity is well shown in the table on p. 578.

In the experiments lasting four hours, the activity was clearly at a maximum in faintly acid solutions.

The maximum is less marked in the case of the experiments lasting 21 hours. The limits within which action takes place are obviously very narrow.

Activity towards α-Methyl Glucoside of Solutions prepared by extracting Dried Yeast with Solutions of various Mixtures of Monopotassium and Disodium Phosphate.

Solution	Sörensen values	Walker values	Glucose formed 4 hours' action	Glucose formed 21 hours' action
			grm.	grm.
β	9·3	200·0	nil	nil
α	8·93 } alkaline	67·6 } alkaline	0·003	0·06
A	8·3	20·0	0·16	0·49
B	7·35	2·3	0·34	0·87
C	6·81	1·55	0·37	0·90
D	6·24	5·8	0·54	0·91
E	5·3 } acid	50·0 } acid	0·47	0·88
F	4·53	300·0	0·31	0·80
G	4·0	1000·0	0·23	0·72
H	3·3	5000·0	nil	nil

Similar results obtained with other enzymes are shown in the following table:—

Enzyme	Hydrolyte	Time in hours	Relative amounts of action in solutions of								
			Alkalinity			Acidity					
			68	20	2·3	1·5	5·8	50	300	1000	5000
Maltase* ...	Maltose	4	1·6	2·2	2·9	3·5	3·4	3·35	3·0	2·9	0·15
Emulsin ...	Salicin	4		0·54	2·6	6·2	9·4	11·0	11·2	10·3	2·0
Aucuba leaf powder	„	5		0·15	9·5	17·3	24·9	24·3	17·0	10·6	nil
„ „	„	24		1·1	30·8	71·1	89·0	87·6	57·2	36·7	„

* The yeast extract used in this experiment was a portion of that used in the experiment with α-methyl glucoside.

[*Note added July* 30.—A particularly instructive series of observations, represented in fig. 5, were made in the course of our experiments with urease. They illustrate the remarkable sensitiveness of the enzyme to acid and alkali.

The action of the enzyme on urea alone is represented by Graph 1. Graph 2 represents the action in presence of M/25 monopotassium phosphate, Graphs 3 and 4 representing the effect produced by M/5 and 2M/5 solutions of this salt. It will be noticed that the smallest proportion produced an acceleration of the change from the outset; the intermediate proportion at first caused a retardation, but the action soon set in at a greater rate than was observed in the case of the smaller proportion. In the case of the highest proportion, the retardation was at first very great, but it is obvious that recovery then set in. The effect of the alkaline salt, M/5, Na_2HPO_4, was prejudicial from the beginning.

It was noticed that the solution became cloudy on mixing the enzyme with the strong solution of the acid phosphate but that it afterwards became clear. Evidently the enzyme was at first in large part neutralised and " coagulated " but as alkali was formed it became redistributed and as the acid phosphate served to neutralise the alkali produced in the change the enzyme preserved its activity to a greater extent than it did when no phosphate was present.]

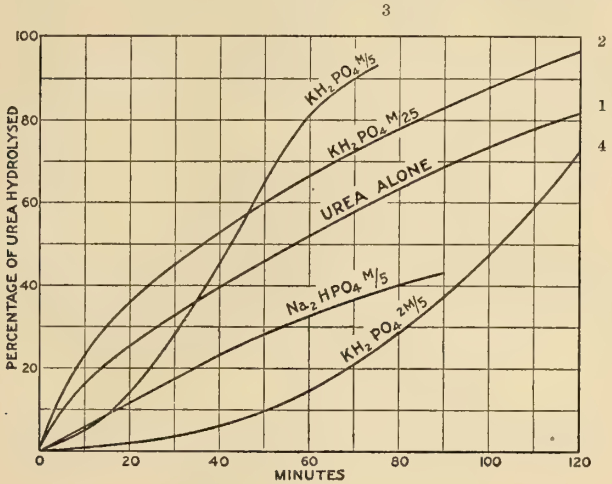

Fig. 5.

Influence of the Products of Change and of other Substances.—In these studies, stress has always been laid on the influence which certain of the products of change exercise in retarding the action of an enzyme and the special influence of one or the other of the products of change has often been advanced as an argument of consequence in favour of the conclusion that the enzymes are strictly specific agents.

This, in fact, is to be regarded as a principal argument in favour of the hypothesis now advocated and we have therefore been led to reconsider very carefully the evidence brought forward in this connexion at an early stage of this inquiry. We now desire to amend it in important particulars, having in the interval arrived at the conclusion that it is necessary to be very careful in interpreting the effects produced by various substances and not to regard them always as specific influences.

In the first place, it is necessary to take into account the manner in which the proportion of the hydrolyte present influences the result and to realise that the degree of concentration is soon reached at which an enzyme has maximum activity.

As already remarked, the course of change is in no way that to be expected if the action be a mass action effect.

V. Henri gives abundant proof in his comprehensive memoir* that the activity of enzymes such as invertase, emulsin and diastase falls off as the concentration of the solutions is increased beyond a certain limit—about half volume-normal strength in the case of cane sugar. The observations of all other workers support this view.

In our work on urea, numerous instances are given showing that the enzyme is more effective in the weaker solutions.

The following results obtained with α-methyl glucoside and α-glucase (yeast extract) also afford evidence that, instead of increasing, the activity of the enzyme soon reaches a superior limit and then diminishes as the concentration is increased.

Concentration of glucoside	Weight of glucose produced	
	grm.	grm.
M/2 (9·7 grm. per 100 grm. water)	2·16	2·21
M (19·4 „ „)	2·37	2·35
3M/2 (29·1 „ „)	2·15	2·25
2M (38·8 „ „)		1·98

The diminution in the activity of the enzyme caused by an increase in the concentration of the hydrolyte beyond a certain point is to be set down, we believe, mainly to changes in what may be termed broadly the osmotic state of the solution—to changes in the state of the solvent which affect the state of "hydrolation" both of enzyme and of hydrolyte.

According to the hypothesis advocated in several previous communications, as the concentration of the solution is increased, the extent to which the surfaces of the enzyme and of the hydrolyte are hydrolated effectively must vary and must diminish as the concentration is increased beyond a certain maximum: consequently, the reciprocal activity of enzyme and hydrolyte must vary and must diminish so soon as the degree of concentration is exceeded at which the degree of hydrolation ceases to be that which is most favourable to the occurrence of change.

In no other way does it appear to us to be possible to account for the marked effect which an increase in the concentration of the hydrolyte has in diminishing the rate at which change takes place—the effect being both beyond that which is to be expected to arise from a reversal of the change and producible also by substances which must be inert in this respect. In fact, a similar effect is produced whenever a substance is added which increases the "osmotic tension" in the solution. Moreover, not only enzymes but all hydrolytic agents are affected.

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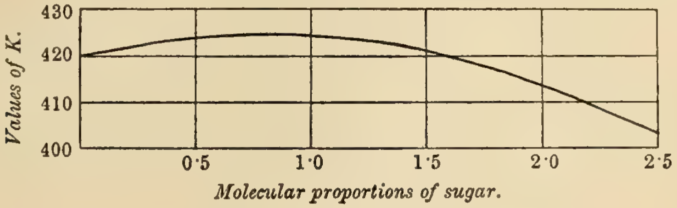
* 'Lois générales de l'Action des Diastases,' Paris, 1908.

Thus Mr. Worley's experiments (S. XII) prove that the rate of hydrolysis of cane sugar by acids is not proportional to the concentration of the former at all strengths of the solution but soon reaches a maximum, as shown in the following graph representing the results obtained with nitric acid in experiments in which the sugar was the only variable :—



These results, which correspond strictly with those obtained when the concentration of the hydrolyte is varied relatively to an enzyme, are of special importance as showing that the diminished activity must be due to an alteration in the osmotic state—not to mechanical causes.

That salts should retard hydrolysis by enzymes is only to be expected on account of the "concentrating" effect they exercise.

Neutral substances generally, however, also exercise an inhibiting effect which not only varies according to the proportions in which they are present but is also the greater the less soluble the substance. As slightly or moderately soluble substances are often formed in cases of enzymic hydrolysis this behaviour is of consequence. Results obtained with cane sugar, illustrating the effect referred to, are given in No. XIII of the Solution Studies and also in Nos. XI and XXV. It is shown that, in the case of a considerable number of neutral substances used as precipitants of salts, the less soluble precipitant is always the more efficient. It is difficult to explain the effect they produce otherwise than by the assumption that in presence of the neutral substance the water becomes more active, in the sense that by their interposition the proportion of hydrone molecules in the liquid is increased: consequently the degree of hydrolation of any substances that may be present is lowered both because the solution is more attractive and the surface therefore less attractive of hydrone and because the neutral molecules also interfere directly at the hydrolated surfaces and promote dehydrolation mechanically. The less they attract hydrone and the more easily they can move about in the liquid, the more active they are apparently: it is on this account that the higher alcohols, chloroform and similar substances are so specially active.

The same explanation may be given of the effect produced by neutral substances in reducing the electrolytic conductivity of solutions. Moreover, it has been shown in S. XX and XXVI that chemical changes are similarly influenced. Thus, in the former, it is proved that the rate at which urea is formed from ammonic cyanate is influenced by alcohols of the ethylic series and more promoted by the higher slightly soluble alcohols than by the lower easily soluble. In the latter, it is shown that the equilibrium between the two isodynamic forms of fructose is affected by a variety of neutral substances and that the effect produced is greater the less soluble the substance.

Preferential Retardation of the Activity of Enzymes by Compatible Materials.—The effect of various substances on the hydrolysis of α-methyl glucoside by α-glucase is shown in the following table:—

	Weight of glucose liberated		Percentage of glucose hydrolysed
	Exp. I	Exp. II	
	grm.	grm.	
M/5 α-methyl glucoside	0·97		
" + M/5 α-methyl glucoside	0·945	0·99	
" + M/5 glucose	0·2	0·2	
" + M/5 galactose	0·82	0·8	
M/5 α-methyl glucoside (3·6 grm. per 100 grm. water)	2·03		57·2
(7·2 " ")	2·69		37·3
" + M/20 glucose	1·67		46·5
" + M/10 "	1·40		39·0
" + M/5 "	0·94		26·0
" + M/5 galactose	1·47		41·0
" + M/20 saligenin	1·47		40·8

It will be noticed that glucose has an effect which is out of all proportion large in comparison with that produced by other materials. Galactose has not nearly so great an effect though its inhibiting power is considerable in comparison with that of α-methyl glucoside, which is practically without influence.

The following results obtained with the β-glucoside salicin and the enzyme emulsin are in harmony with the above conclusions:—

	Percentage hydrolysed		
	Little enzyme	Much enzyme	
M/10 salicin	28·3	88·8	85
„ + M/10 glucose	20·8	78·7	77
„ + M/20 saligenin	23·5	84·0	80
	Glucose obtained		
	grm.		
M/10 salicin alone	0·388		
„ + M/10 α-methyl glucoside	0·385		
„ + M/5 „	0·368		
„ + 2M/5 „	0·364		
„ + 4M/5 „	0·331		
Complete hydrolysis	0·450		
M/10 salicin	1·52		
„ + M/10 α-methyl glucoside	1·50		
„ + M/5 „	1·44		

It will be noticed that both glucose and saligenin have the greater effect when the amount of enzyme present is small. It is very noteworthy that saligenin has so marked an effect: but both salicin and saligenin are slightly soluble substances and it is to be expected therefore that the former would be specially sensitive. We do not regard the influence on the activity of emulsin exercised by saligenin as in any way a case of preferential retardation by a compatible material—both on general grounds and because it has so marked an influence both on α-glucase and on urease.

The influence of the small quantities of alcohol produced during hydrolysis is subordinate, although at a higher concentration the alcohol soon exercises a marked influence. The following tables show the magnitude of this effect:—

M/10 α-Methyl Glucoside + Yeast Extract.

Medium	Relative change	Medium	Relative change
Water	90	Water	91
10 p. c. ethylic alcohol	44	10 p. c. methylic alcohol	51
20 „ „	25	20 „ „	21
40 „ „	10	40 „ „	0
60 „ „	2	60 „ „	0

Methylic alcohol is apparently more toxic than the higher homologue.

2 per cent. Salicin + Emulsin.

Medium	Relative change
Water	86·0
20 p. c. ethylic alcohol	38·0
40 " "	14·0
60 " "	10·0
80 " "	4·8
90 " "	2·0
95 " "	0·2

The enzyme is largely precipitated from yeast extract when 40 per cent. of alcohol is present. Emulsin is also largely precipitated from a solution of this strength but the enzyme remains active as a hydrolytic agent, even in very strong alcoholic solution; attention has recently been drawn to this fact—of which we have long been aware—by Bourquelot.

The manner in which the rate at which α-methyl glucoside is hydrolysed is affected over a considerable period by glucose is shown in the following table and in the corresponding graph.

Action of α-Glucase on α-Methyl Glucoside Alone and in Presence of Glucose.

Time	Weight of glucose formed	
	Glucoside alone	Glucoside + glucose
	grm.	grm.
2 hours	0·43	0·15
3½	0·66	0·24
5	0·85	0·29
7	1·03	0·37
8½	1·21	0·41
10	1·31	0·45
24	1·82	0·82

The slight influence exercised by α-methyl glucoside on the hydrolysis of salicin may be attributed entirely, we think, to its concentrating or dehydrolating effect, in view of the inappreciable effect produced by an equivalent amount and the gradual increase of the effect as the concentration is raised.

In our former experiments, E. III,[*] the solutions used were highly concentrated. Thus, 10 grm. of β-methyl glucoside, together with 10 grm. of maltose, were dissolved in water to 100 c.c. (including the enzyme). Under these conditions, the action of the α-enzyme was retarded by the β-glucoside :

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[*] 'Roy. Soc. Proc.,' 1904, vol. 73, p. 516.

similar results were obtained with emulsin and milk sugar, showing that *a*-methyl glucoside controlled the action. Our recent observations recorded above show no such effect in the case of dilute solutions. We therefore think that our former conclusions were erroneous, as they were based on results obtained with solutions so concentrated that the point at which the enzyme has maximum activity was exceeded and, consequently, the addition of any substance would cause a retardation of hydrolysis.

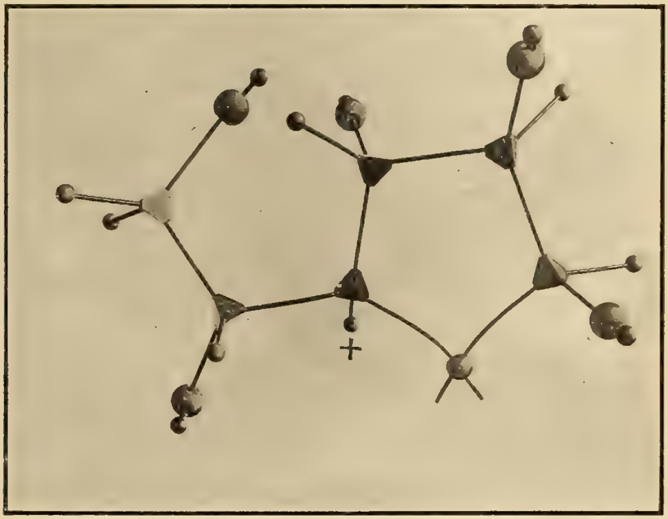

Fig. 6.—Glucose.

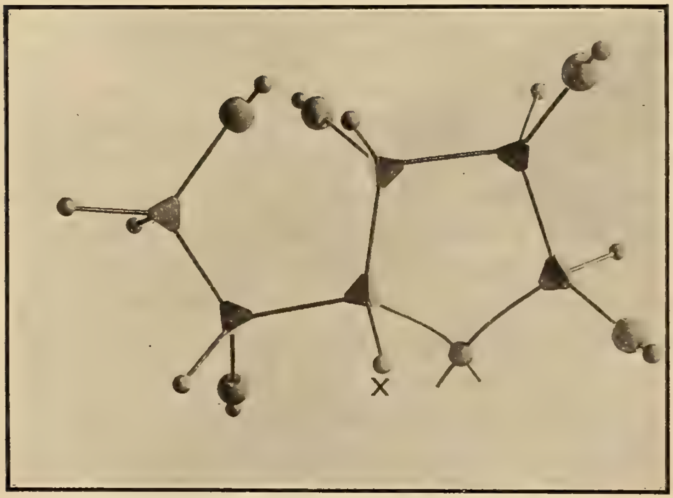

Fig. 7.—Galactose.

It remains to consider the distinct effect produced by galactose.

On reference to the diagrams figs. 6 and 7 representing the spatial arrangement of the atoms in the two sugars, it will be noticed that the only difference between them is that the hydrogen atom X shown to the left of the oxygen atom in the ring is in a plane behind the ring in the one case, and in a plane in front of the ring in the other; there is also a corresponding difference in the relationship of the linking oxygen atom to the ring plane. The difference between the close packed assemblages, therefore, would probably be small: though sufficient perhaps to reduce the compatibility of the two molecules, some degree of compatibility might still persist.

This is one of those cases of minute difference which it will be important to study further, especially in view of the observation made by more than one worker that some yeasts "acquire" the power of fermenting galactose if habituated to its presence. The question of the presence of a distinct enzyme in emulsin capable of hydrolysing milk sugar and presumably of inducing the synthesis of β-galactosides must also be reconsidered from this point of view: we are at present engaged in this inquiry.

Studies on Enzyme Action. XXI.—*Lipase* (III).

By H. E. Armstrong, F.R.S., and H. W. Gosney. B.Sc.

(Received June 13,—Read June 26, 1913.)

On account of the part which Lipase plays in promoting the resolution of fats generally into fatty acid and glycerol, one of the most important processes in animal nutrition, it is desirable that a clear picture should be obtained of the manner in which the activity of the enzyme is exercised.

The material hydrolysed—the fat—being practically insoluble and the enzyme presumably a colloid, the interaction to be considered is that of substances insoluble in water and therefore presents unusual features.

Two brief communications on the subject were made to the Society in 1905 and 1906.* In the first of these, which had reference to the enzyme in castor oil seeds, it was stated that Connstein's contention had been confirmed that the presence of acid is necessary to condition the hydrolysis of a fatty oil by the enzyme and that practically any acid was effective provided a sufficient amount were used. As acids did not act equally in

* 'Roy. Soc. Proc.,' B, vol. 76, p. 606; vol. 78, p. 376.

equivalent quantities, although when used in sufficient amount the weak were as effective as the strong, it appeared to be probable that the strength of the acid was a factor in the action.

The enzyme could not be separated from the seed. An attempt that was made to liberate it by treating the residue left after extracting the seed with sulphuric acid and removing the excess of acid by washing thoroughly was unsuccessful: apparently the enzyme was destroyed.

The final conclusion arrived at was that the *Ricinus* enzyme is possessed of properties which make it specially capable of promoting the hydrolysis of glycerides of the higher fatty acids.

In the second communication, results were quoted which again were an indication that the strength of the acid used in promoting the hydrolysis of fats was a factor of importance and that, in the case of the stronger acids used, no action took place when more than a certain proportion of acid was present.

The conclusion of chief importance arrived at in this communication was that comparable results could only be obtained, in the case of ethereal salts soluble in water, by using solutions of equivalent concentration: from which it follows that no comparison can well be made between soluble and insoluble ethereal salts.

When soluble salts were compared, it was found that the enzyme was the more active the less soluble the ethereal salt and the weaker the acid from which the salt was derived. Thus ethylic succinate was hydrolysed to a considerable extent and the far more soluble allied ethylic tartrate (dihydroxysuccinate) was scarcely if at all affected,* the salt of intermediate solubility, ethylic malate (monhydroxysuccinate), being acted upon less readily than the succinate but more readily than the tartrate.

In explanation of these results, it was suggested that the attachment of the enzyme to the carboxylic centre of the ethereal salt—the necessary first act in the process of hydrolysis—was *interfered with by the hydration of this centre*, the implication being that hydration took place the more readily and to a greater extent the more soluble the ethereal salt.

Various results were quoted in this communication showing that, whilst it was less active towards fats than *Ricinus* lipase, liver lipase determined the hydrolysis of various ethereal salts without any addition of acid: though attention was not directed specially to this point, on this account and because of the retardation of hydrolysis by acids when used in excess, the opinion had been formed that the acid served merely to liberate the enzyme from *Ricinus* seed and was not directly effective as a co-enzyme in promoting the hydrolysis of the ethereal salt. In other words, it appeared to be probable that the zymogen in the seed was merely a salt which became active when "neutralised" by an acid.

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* We are inclined to think that the slight amount of action observed in this case is due to the fact that the acid liberated by the direct action of water on the tartrate prevents hydrolysis by the enzyme.

Since 1906, at intervals, many attempts have been made by one of us to arrive at an understanding of the peculiarities of Lipase but it has been possible only recently to interpret the results in a simple and consistent manner, so as to correlate the effects produced by this enzyme with those observed in the case of other natural hydrolysts: in fact until the views which are put forward in the previous communication had taken definite shape and a clear conception had been formed of the manner in which enzymes generally effect hydrolysis, it was difficult, if not impossible, to formulate an explanation of the manner in which an enzyme operates when acting under such peculiar conditions and to appreciate the relative significance of the various observations made with Lipase.

Preparation of the Enzyme.—The most important advance made in recent years in connexion with Lipase is the discovery by Yoshio Tanaka* that an active enzyme may be prepared by digesting pressed castor oil seed with a proper amount of acid, then washing to remove all soluble matter. This material is most active in a neutral medium, less so in the presence of acid, especially mineral acid.

In a more recent communication, published in September 1912,† it is stated that the optimum quantity of acid is 5 c.c. of N/10 sulphuric or 6–7 c.c. of N/10 acetic acid for each gramme of the pressed seed. The method recommended is to digest 100 grm. of pressed or extracted castor oil seed with 600–700 c.c. of the acetic or 500 c.c. of the sulphuric acid at 30–35° and after about 30 minutes to wash the residue thoroughly with water and then dry the pasty mass at a temperature not exceeding 40°. If oil be digested at 30–35° with 3–4 per cent. of the dried Lipase powder thus prepared and 6–10 times as much water as powder, about 90 per cent. of the glyceride is hydrolysed within 7–10 hours.

In our experience, the enzyme prepared with a very weak acid such as acetic is distinctly superior to that obtained when a stronger acid is used: the enzyme appears to be in some way altered by "fixation" of the acid and the effect cannot be counteracted by neutralisation with alkali.

We have usually prepared the enzyme by crushing the shelled castor oil seed in a mortar and digesting the oily mass with petroleum spirit: after 24 hours, the greater part of the oil is removed by squeezing it through calico cloth. The residue is treated twice in the same manner, using ordinary ether instead of petroleum spirit; it is then ground up in a mortar and digested during about 15 minutes with 80 c.c. of N/10 acetic acid to every 10 grm. of the meal. The liquid having been filtered off, the residue is washed several times by alternately transferring it to a beaker containing water and pouring off the liquid through a filter: it is then dried in a vacuum desiccator and, when dry, ground up and sifted through fine muslin. Succinic acid can be substituted for acetic but when tartaric acid is used the product is less active. During the washing process about 40 per cent. of the material goes into solution. The amount of powder obtained is about 9 per cent. of the weight of the seeds. Our preparation has not proved to be so active as that described by Tanaka but we have obtained better results with it than with a preparation made according to his directions.

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* 'Journ. of the College of Engineering, Tokyo Imperial University,' 1910, vol. 5, No. 2, p. 25.

† *Ibid.*, No. 4, p. 125.

Experimental Method.—The hydrolytic experiments were all carried out in 50 c.c. Jena hard glass flasks closed with rubber stoppers. These were maintained at a constant temperature in a Hearson incubator, 14 by 12 by 12 inches, heated electrically and provided with an electrically controlled regulator.

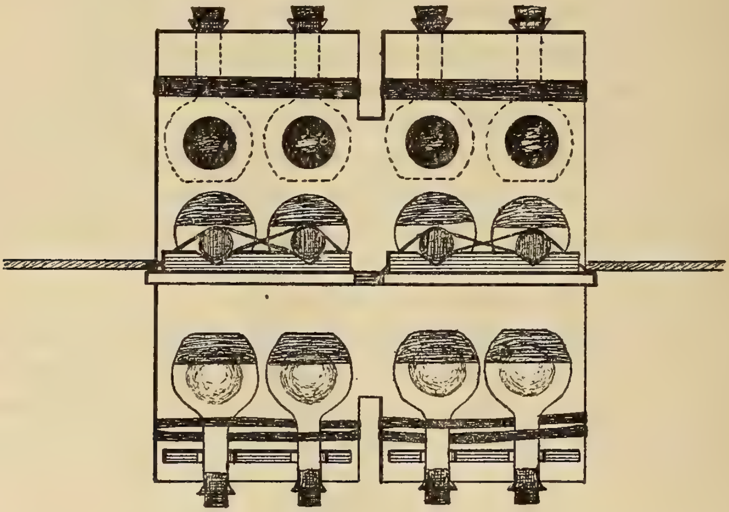
In order to agitate the contents of the flasks during the experiments, they were fixed by rubber bands to paddles carried on a central steel shaft, ⅜ inch in diameter, passing through the horizontal axis of the chamber; this shaft was rotated at a rate of about six revolutions per minute by means of a small motor outside the chamber. The shaft carries a square metal block attached to which are four brass fins at right angles to each other. A wooden plate, 4⅝ by 10 inches, about ¼ inch thick, forming the paddle, is screwed to each of the brass fins; a narrow strip of wood is fixed near the edge of the paddle and grooves are cut in this to receive the necks of the flasks; the bodies of the flasks rest in corresponding holes cut in the paddles. The arrangement will be obvious from the figure on p. 590.

The titrations were carried out in 50 per cent. alcoholic solutions, using normal or N/10 alkali and phenolphthalein.

Acidity of the Enzyme.—The untreated oil-free seed residue is distinctly acid, neutralising from 2 to 4 c.c. of N/10 alkali per gramme; whilst it hydrolyses succinic ether nearly as well as the acid treated material, it has very little action on fatty oils.

When either acetic or succinic acid is used and the meal is washed until the filtrate is no longer acid to phenolphthalein, the product is still acid, that prepared with the aid of acetic acid requiring from 4 to 5 c.c. of N/10 alkali per gramme to neutralise it; when tartaric acid has been used, as much as 7–8 c.c. of alkali may be required; the product obtained with the aid of sulphuric acid has a still higher acidity and is also less active.

Very little if any acetic acid is absorbed during the treatment but the amount of tartaric acid fixed is considerable: thus in one experiment, using 5 grm. of the residue, on titrating the washings, it was found that 15 out of 40 c.c. of N/10 acid were retained: on using N/10 sulphuric acid, 22·5 out of 40 c.c. were absorbed.



Effect of Acid on the Activity of the Enzyme.—The following results were obtained on contrasting the action on 10 c.c. of M/20 solution of succinic ether, to which different amounts of glycine had been added, of 0·5 grm. of enzyme prepared with the aid of tartaric and acetic acid respectively: in the latter case, two different preparations were tested.

Molecular proportions of glycine	Percentage hydrolysed by enzyme prepared with tartaric acid	Percentage hydrolysed by enzyme prepared with acetic acid	
		a	*b*
0	42·2	58·5	50·8
½	42·7	57·6	51·4
1	46·5	61·3	53·3
2	48·5	63·5	56·9
3	—	67·0	
4	58·5	—	62·6
5	—	70·2	
6	64·4	—	68·0

These results show that the recovery of activity, even in presence of a considerable excess of the "basic" material, is only moderate; they appear to justify Tanaka's conclusion that the enzyme is more active under neutral than under acid conditions.

The inferiority of the tartaric product is equally obvious when tested by means of oil. Thus on digesting 0·5 grm. of the powder with 5 grm. of olive oil and 2 c.c. of water during 20 hours, the amounts hydrolysed were as follows:—

	Enzyme *a* prepared with acetic acid	Enzyme prepared with tartaric acid
(1)	77·6 per cent.	45·1 per cent.
(2)	75·5 ,,	48·4 ,,

To test the effect of concentration, three 5 grm. samples of oil-free meal were digested with equal volumes (40 c.c.) of solutions of tartaric acid of different strength (N/5, N/10, N/40) and after washing and drying, 0·5 grm. of each product was digested with 10 c.c. of a solution of succinic ether. The amount of hydrolysis effected in 20 hours was:—

Untreated enzyme	51·1 per cent.
Digested with N/5 acid	30·9 ,,
N/10 ,, ...	41·5 ,,
N/40 ,, ...	8·5 ,,

As it was possible that insufficient acid had been used in the case of the N/40 solution, a constant amount of tartaric acid but different volumes of the solution were used.

Enzyme prepared with the aid of	20 c.c. N/5	acid hydrolysed	42·6	per cent.
	40 ,, N/10	,,	43·9	,,
	80 ,, N/20	,,	54·4	,,
	160 ,, N/40	,,	46·1	,,

The following two tables afford further illustrations of the influence of acids:—

Table showing the Effect of increasing Amounts of various Acids on 0·5 grm. of treated Enzyme acting during 20 hours on 10 c.c. M/20 Solution of Succinic Ether.

Concentration of acid	Glycine	Butyric	Succinic	Malic	Tartaric	Sulphuric
No acid	58·5	56·5	60·3	58·7	60·7	55·7
N/40	57·6	60·5	56·7	60·3	58·8	8·1
N/20	61·3	61·8	55·8	—	52·6	
N/10	63·5	57·3	55·0	56·6	33·3	
N/5	70·2	44·2	55·6	51·1	5·2	
N/3·3	—	17·0	53·6	21·2	—	

Table showing the Effect of the Presence of Four Equivalents of various Acids on the Action of 0·5 grm. of treated Enzyme on 10 c.c. M/20 Solution of Succinic Ether, *i.e.*, in N/5 Acid Solutions.

	Percentage hydrolysed
Glycine	71·0
Acetic acid	63·4
Succinic acid	62·0
No acid	62·0
Butyric acid	50·9
Malic acid	41·3
Citric acid	29·6
Tartaric acid	15·8
Sulphuric acid	0·8

To hydrolyse succinic ether, a relatively large amount of the enzyme is required. Thus on digesting 1 grm. of the Lipase powder with 10 c.c. of an M/20 solution of ethylic succinate (0·087 grm.) during 48 hours, the ethereal salt was completely hydrolysed: but ½ grm. of the powder was insufficient.

The enzyme is only slightly, if at all, affected by the action and will hydrolyse fresh ester but its action on oil is somewhat impaired. Thus when 0·5 grm. of enzyme was allowed to act during 20 hours on four different quantities, each 10 c.c. M/20 ethylic succinate, in succession, hydrolysed

52·0	per cent.	during	1st	use
50·5	"	"	2nd	"
48·4	"	"	3rd	"
47·6	"	"	4th	"

The once used enzyme, however, caused only 29·5 per cent. hydrolysis of 5 grm. of oil, the fresh enzyme causing about 66 per cent. in the same time. This lipoclastic weakening of the enzyme is possibly due to a loss of emulsifying power.

Whatever the amount of enzyme present, the amount of action appears to be approximately proportional to the amount of ethereal salt in solution. Thus 0·5 grm. enzyme acting during 20 hours at 30° C. hydrolysed:—

0·0096	grm. ethereal salt	in	10	c.c.	M/100	solution
0·0234	"	"	10	"	M/50	"
0·0327	"	"	10	"	M/33·3	"
0·0487	"	"	10	"	M/20	"
0·0710	"	"	10	"	M/15	"
0·0913	"	"	10	"	M/10	"

But apparently, when undissolved ethereal salt is present, the amount of action is not greatly in excess of that effected in saturated solution. Thus, on digesting 5 grm. of succinic ether with 5 c.c. of water and 0·5 grm. of enzyme during 20 hours, only 0·205 grm. was hydrolysed—or about twice as much as when an M/10 solution was used.

When the amounts of ethereal salt and enzyme were kept constant the amount hydrolysed decreased as the solution was diluted.

Thus in 5 c.c. M/10 solution +	0 c.c. water	62·5 per cent.	was hydrolysed
	2 ,,	59·4 ,,	,,
	5 ,,	61·2 ,,	,,
	10 ,,	53·5 ,,	,,
	20 ,,	40·1 ,,	,,
	40 ,,	15·4 ,,	,,

Thus everything tends to show that lipase is very sensitive to the action of acids, though acids are produced by its action. Its inferiority as a hydrolyst of ethereal salts other than fats and its power of hydrolysing the complex natural glycerides readily, apparently to an almost unlimited extent, would seem to be due to the fact that the acids that are liberated from fats are scarcely if at all soluble in water and very weak.

Mode of Action of Lipase.—The argument previously put forward (Part II) in explanation of the activity exercised by lipase is as follows:—

"The ethereal salts which are hydrolysed under the influence of lipase are all compounds of the type R′.CO.OX′. Since R′ and X′ may be varied within wide limits, it cannot well be supposed that the selective action of the enzyme is exercised with reference either to R′ or X′; consequently, the controlling influence must be attributed to the carboxyl radicle (CO.O): the enzyme must be so constituted that it can 'fit itself to this group.'

"Our experiments have led us to form the provisional hypothesis that the hydrolysis of the ethereal salt by Lipase involves the direct association of the enzyme with the *carboxyl centre*, and that such association may be prevented by the 'hydration' of this centre: consequently, that those salts which are the more attractive of water will be the less readily hydrolysed."

The behaviour of soluble ethereal salts, especially the fact they are the less readily acted upon the more soluble they are and the stronger the acid from which they are derived, may be better accounted for, however, by assuming *not* that the carboxylic centre of the ethereal salt is hydrated and that therefore the association of the enzyme with this centre is *prevented*: but that salt and enzyme are the more kept apart, through the agency of water, the more soluble the salt is, because the salt is the more attracted by the water; and that the enzyme is the more interfered with, through the fixation of acid, the stronger the acid.

It remains to account for the special affinity of lipase to fats. Owing to the fact that it acts on carboxylic ethereal salts in general, it cannot be supposed that it has any special attractive influence over the molecule of the hydrolyte as a whole, such as is pictured in the previous communication as exercised by the enzymes which hydrolyse glucosides, for example, though the fact that it acts preferentially on fats cannot be left out of account. It would seem to be necessary to assume only that the enzyme is one in which an attractive carboxylic centre is freely exposed.

To judge from the properties of acids generally, it would seem that the carboxylic group has a marked tendency to combine with itself—thus acetic acid appears to exist under ordinary conditions as a polymerised form of the fundamental molecule $CH_3.CO.OH$; and, judging from their slight solubility, this is true also of a great many acids. The *free* carboxyl radicle, CO.OH, probably has marked attractive power and far greater activity than is apparent in acetic acid, for example.

It is a question, therefore, whether the properties of lipase may not be accounted for on the assumption that it is a colloid molecule possessed of a carboxylic or even a phosphoric group so situated that it cannot be self-neutralised but yet sufficiently near to a basic centre to be interfered with by any acid which can combine with this latter.

It may well be that the configuration of the enzyme is such as specially to favour its association with glycerides of the higher fatty acids. But the association of enzyme and hydrolyte is doubtless determined by an intervening water film, *i.e.* the carboxylic centres in the two compounds are both to be thought of as hydrolated and as brought into contact through the agency of the attached water molecules. The number of molecules thus activated will depend on the osmotic conditions which prevail in the mixture undergoing change.

We are under the impression that the lipase powder contains an emulsifying constituent and that its activity is perhaps in no small measure dependent on this constituent. It is impossible to say at present whether the intrinsic acidity of the powder prepared by means of a weak acid which is not retained to any appreciable extent is that of the enzyme proper or of a practically insoluble acid associated with it: we are inclined to think that the intrinsic acidity of the powder is to be correlated with its emulsifying power. As the decrease in the activity of the enzyme when it has a high acid value is equally marked towards succinic ether, however, the inferiority cannot well be attributed solely to loss of emulsifying power.

Influence of the Products of Change.—Under ordinary conditions the hydrolysis of fats by lipase powder is incomplete—partly perhaps because the action is reversible but mainly, we think, on account of the retarding influence of the products of change and the decay of the enzyme.

With regard to the influence of the acid liberated from a fat on the course of change, the conclusion arrived at recently by Tanaka will be obvious from the following quotation :—

"Different amounts of soya bean oil were hydrolysed by the 'lipase powder' in the presence of different amounts of fatty acid at 38° C. The results were as follows:

Oil	Acid	Enzyme	Water	Grammes of oil hydrolysed		Percentage of oil hydrolysed	
				1 hour	2 hours	1 hour	2 hours
grm.	grm.	grm.	grm.				
50	0	2	18	22·00	32·50	44·0	65·0
40	10	2	18	17·73	26·33	44·1	65·5
30	20	2	18	13·13	20·31	43·2	66·8
10	40	2	18	4·70	7·33	43·5	67·9

"These data show that the amount of oil hydrolysed is directly proportional to the amount of oil present or the rate of change follows the law of mass action, even in the presence of a large amount of free fatty acid. This result proves without doubt that the fatty acid has no retarding effect upon the enzyme action, because, if that were the case, it would lead to a slowing of the reaction greater than that due to the diminished concentration of the neutral oil."

Tanaka's experiments do not appear to us to justify these conclusions. It will be noticed that the four interacting substances were all present in different *relative* proportions in each of the four experiments—consequently the results are not comparable.

It is not to be expected that an oil will behave as an aqueous solution, but there must be some optimum proportion of enzyme to oil beyond which the oil will be in excess, as it is obvious that any influence the products of change can exercise will be relatively greater the smaller the proportion of oil present.

That the extent to which hydrolysis takes place is not proportional to the amount of oil used is shown by the following results of a series of experiments in which 1 grm. of enzyme and 4 c.c. of water were digested during 20 hours with varying amounts of oil:—

Oil used	No. of c.c. N/NaOH neutralised	Per cent. of oil hydrolysed
10 grm.	18·6	54·5
20 „	26·3	38·1
30 „	28·9	28·0
50 „	35·6	20·6

In a second experiment in which 25 c.c. of water was used together with 1 grm. of enzyme, the results were as follows :—

10 grm.	21·1	61·8
20 „	29·6	43·3
30 „	33·8	32·8
50 „	39·8	23·2

It will be noticed that in both cases only about twice as much oil was hydrolysed when the amount used relatively to a given weight of enzyme was increased five times.

The amount of oil hydrolysed is also not proportional to the amount of enzyme used: thus on digesting 5 grm. of oil and 30 c.c. of water at 30° C. during three days the percentage hydrolysed was :—

By 0·5 grm. enzyme	58·5
„ 1·0 „ „	83·0
„ 1·5 „ „	92·0

Apparently, when sufficient water is present, an increase in the amount produces relatively little effect. Thus on digesting 10 grm. of oil with 1 grm. of enzyme and varying amounts of water during 20 hours, the results obtained were as follows :—

Water used	Percentage hydrolysed
3 c.c.	88·2
5 „	90·3
10 „	90·5
25 „	90·1
50 „	89·7

Oleic acid is strong enough to make the untreated enzyme active if used in sufficient amount. Thus on digesting 5 grm. of oil, 2 c.c. of water and 0·5 grm. of oil-free seed with varying amounts of oleic acid, the results obtained were :—

Molecular proportion of oleic acid	Percentage hydrolysed
0	1·6
0·5	3·2
1	5·8
2	23·4
4	32·8
6	35·7

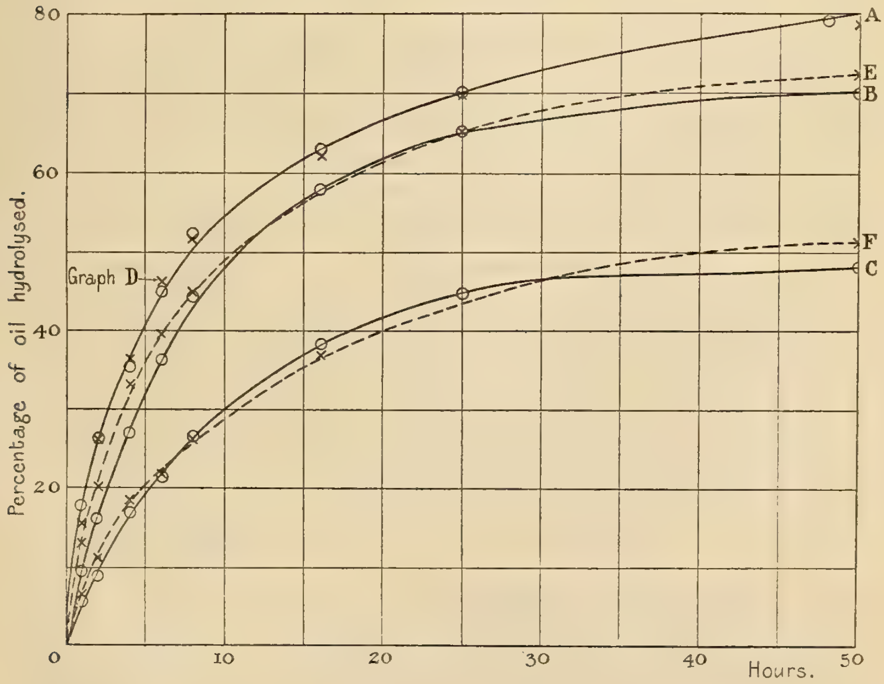
But oleic acid has a marked retarding effect. Thus in 20 hours the percentage of oil hydrolysed in presence of different proportions of the acid by the enzyme prepared with the aid of acetic acid was as follows :—

0	80·6
0·5	82·4
1	84·3
2	70·9
4	58·1
6	45·6

The retarding effect of oleic acid is shown more clearly in the following table and diagram representing the results obtained in experiments in which the rate of change was contrasted in presence of various proportions of one or other or both products of change :—

Table showing Percentage of Change effected by 0·5 grm. of Enzyme acting on 5 grm. of Olive Oil and 2 c.c. Water at 30° C. (O = 1 molecular proportion of Oleic Acid; G do. Glycerol.)

Time in hours	Oil alone Graph A	Oil + 3 O Graph B	Oil + 6 O Graph C	Oil + G Graph D	Oil + 2 G Graph E	Oil + 3 O + G Graph F
1	17·9	9·5	5·6	15·7	13·0	6·6
2	26·3	16·1	8·9	26·1	20·1	11·3
4	35·4	27·0	16·9	36·4	33·1	18·5
6	44·9	36·4	21·2	46·1	39·6	21·8
8	52·3	44·2	26·3	51·6	44·8	26·1
16	62·8	57·9	38·2	61·9	57·2	36·8
25	70·1	65·2	44·5	69·8	65·2	44·5
50	78·9 (48 hours)	69·7	47·7	78·3	72·2	51·1



We are inclined to attribute the slight effect glycerol produces to alterations in the osmotic conditions affecting the enzyme-oil interface. On the other hand, we regard the powerful retarding effect of oleic acid as a case of the preferential retardation referred to in the previous communication, this being promoted by the solubility of the acid in the oil; apparently, it is too weak an acid to interfere as such.

As it is conceivable that the reduction of the rate of hydrolysis in presence of the acid may be due in part at least to the effect it has in promoting the reversal of the interaction, we have endeavoured to ascertain if the change can be carried to completion by removing the glycerol as it is formed. To this end, the hydrolysis was carried out in a cell constructed by stretching parchment paper over the opening at the narrow end of a small bell-jar. An emulsion of 50 grm. of oil, 20 c.c. of water and 5 grm. of enzyme was placed within the jar, which was surrounded with water; the oily mixture was constantly stirred by means of a glass stirrer actuated by a fan kept in rotation by means of a small flame.

After about 24 hours, a curd or clot separated from the mixture and only 67·7 per cent. of the oil was hydrolysed after three days. Apparently the enzyme had been either destroyed or in some way modified and rendered inactive. This result was confirmed in a second experiment in which the mixture was kept at rest.

Observers differ as to the effect of use on lipase. It appears to be easily changed if left in contact with water and this probably is the reason why different preparations differ more or less considerably in activity. The following results may be quoted in this connexion.

1·5 grm. of lipase powder was shaken up with water and the mixture left at 30° C. during two days in a diffusion thimble immersed in water. As a control, 1·5 grm. of the powder was merely digested with the same volume of water in a flask. Toluene was added in each case. The water in the beaker remained clear during 24 hours but was cloudy after 48 hours. The solid was then filtered off and its action tested as follows.

A fourth part of each enzyme preparation was added to 5 grm. of oil and portions of the water used for dialysis, etc., as given in the following table, water being added to make up the volume to 18 c.c. in each case:—

	Percentage hydrolysed in 24 hours
1. Untreated acid prepared enzyme	36·3
2. Enzyme from diffusion thimble	6·1
3. Ditto + liquid from outside of cell	4·6
4. Ditto + liquid from inside cell	9·1
5. Enzyme merely digested with water	8·3
6. Ditto + water with which it had been washed	8·8

But the activity of the enzyme seems also to deteriorate during its action on oil, as the following results show:—

	Oil hydrolysed Grammes
5 grm. of oil + 2 c.c. of water + 0·5 grm. of lipase powder digested during 5 days at 30° C.	*a.* 3·50 *b.* 3·49
As in *a* and *b* but after 48 hours' digestion 5 grm. of oil + 2 c.c. of water were added and the digestion extended to 5 days	*c.* 3·66 *d.* 3·88

Evidently the enzyme had been to a large extent destroyed during the first period of digestion in experiments *c* and *d*.

In view of the possibility that the enzyme does not deteriorate when used to hydrolyse succinic ether in the way that it does when used with a fatty material might be due to the fact that the acid liberated, in the former case, is much stronger than the higher fatty acid and therefore serves to preserve the lipase from destruction by inhibiting the action of proteoclastic enzymes, a second similar series of experiments was made, using 10 c.c. of water in the one set and 10 c.c. of N/10 succinic acid in the other. Practically the same results were obtained with oil and water alone as before but only about one-third as much oil was hydrolysed in presence of the acid and there was no increase after adding the second portion of oil.

What has been said in this section will serve to show that the difficulties attending the study of the action of lipase on fats are considerable. We may here point out that we are inclined to attribute the observed inferiority of liver lipase as a hydrolyst of fats to the unnatural conditions under which it is applied when the material used is the expressed juice from an animal organ. Under natural conditions the lipase and fat are in close conjunction, not suspended in water.

When the graphs given in the diagram on p. 597 are inspected, it is obvious that at first the action proceeds at a rapid rate. Therefore, taking into account the influence of the products of change and the fact that the enzyme diminishes in activity during use, it is not improbable that far from being in accordance with the law of mass action, the change proper proceeds in the manner which appears to be characteristic of other enzymes—*i.e.* the graphs representing the rate of change would be nearly linear, if the disturbing influences could be allowed for.

As to the nature of the enzyme, it is difficult to formulate any definite conception but we are inclined to extend the argument advanced in the previous communication and to take the view that, as already suggested, the configuration of lipase is such as to favour its association with glycerides of the higher fatty acids—in other words, that it contains a glyceric nucleus attached to a carboxylic centre in proximity to an acidic group which can determine the hydrolysis of a fatty molecule which becomes attached to the glyceric nucleus. It is conceivable that a single carboxylic centre in conjunction with the glyceric nucleus would be sufficient to constitute an *acceptor* of fats; this would allow of the attachment of the glyceric nucleus to a colloid complex and leave the third carbon atom free to combine in some other way: if a phosphoric residue became associated with this carbon atom, it would probably serve as *agent*. Such an assumption is in accordance with the instability of the enzyme, it may be added.

Summary.

Following directions given by Yoshio Tanaka, a method is described of rendering the lipoclastic enzyme in castor oil seed active by treatment with dilute acid—preferably acetic acid.

It is suggested that the "zymogen" is merely a salt.

Much evidence is quoted showing that the activity of the acid treated enzyme is interfered with even by dilute acids and that it is easily rendered inert by excess of acid.

It is contended that lipase is specially fitted to hydrolyse the oily glycerides of the higher fatty acids and is not suited to act in aqueous solutions. The interaction must be supposed to take place at and between surfaces separated only by a thin film of water at most.

It is shown that the products of change, both the fatty acid and the glycerol, especially the former, inhibit the interaction of enzyme and oil.

As the rate at which interaction takes place is dependent presumably on the conditions at the colloid surface and these cannot be expressed in terms of the concentration of the solution, it is impossible to apply the law of mass action to the interpretation of the changes observed. Probably, as in other cases of enzymic action, a given amount of enzyme changes equal amounts of material in successive equal intervals of time, the observed departure from this rate being due to the inhibiting effects of the products of change and also to the destruction of the enzyme.

OBITUARY NOTICES

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Lister

LORD LISTER, 1827–1912.

Joseph Lister was born at Upton in Essex on April 5, 1827, and, like many other men who have attained great eminence, belonged to a Quaker family. His father, Joseph Jackson Lister, who was in business in London, occupied his leisure time in scientific researches, more especially in researches in connection with the perfection of the microscope; indeed, it is to his researches that we owe the final perfection of the achromatic lens, which has proved such an essential instrument for microscopical work. He was a man of great accuracy of thought, and a hard worker, and his influence on Lord Lister's character and career was very profound; indeed, Lord Lister himself was never weary of stating how much he owed to his father's early training. It may be noted, in passing, that his father was himself a Fellow of the Royal Society, and that his brother and one of his nephews have also received that honour.

Lister's medical training commenced at University College, and while there he came under the influence of Sharpey, who was then Professor of Physiology, and of Thomas Graham, who was Professor of Chemistry. Sharpey especially exercised great influence in directing his thoughts to the study of physiological problems, which ultimately formed the basis of his great life work; indeed, while still a student he made observations on the contractile tissue of the iris which attracted a considerable amount of attention among physiologists, both in this country and abroad, and he followed that up by work on the muscular tissue of the skin. Both these papers were published in the 'Quarterly Journal of Microscopical Science,' in 1853.

His career at the London University was a very distinguished one, and in 1852 he took the Degree of M.B. Towards the end of his time at University College he became House Surgeon to Mr. (afterwards Sir John) Erichsen, and House Physician to Dr. Walshe. It was more especially while he was House Surgeon that his attention became concentrated on the calamitous results which followed wounds, both operative and accidental, and the terrible cases which he had to deal with riveted his attention, even at that early period, on the subject to which he afterwards devoted the best part of his life.

After he had finished his studies at University College, and before deciding on his future career, he was advised by Dr. Sharpey to visit Edinburgh and see the practice of Prof. Syme, who was then one of the most distinguished surgeons of the age. Towards the end of 1852, therefore, he went to Edinburgh on what he intended to be a short visit. In Edinburgh he very quickly became an ardent admirer of Prof. Syme, who exercised upon him an influence and fascination which were never lost. He constantly quoted Mr. Syme's work and opinions up to the very end of his active surgical work. At first he was simply a visitor in Mr. Syme's wards, but in order to acquire more familiarity with his work and views, he became a dresser, and subsequently his House Surgeon, a post which he occupied for over a year. He then decided to settle in Edinburgh, and in 1856 he was appointed Assistant Surgeon at the Royal Infirmary; at the same time he began to give lectures on surgery in the Extramural school. In preparing these lectures he found that the subject of inflammation and the changes which occurred in the tissues were so imperfectly understood that he made it his first duty to investigate the matter for himself. The results of these researches were of the greatest value in his subsequent work on the treatment of wounds, and enabled him to understand and follow up many things which occurred in wounds where sepsis was excluded, and to make deductions which were of great importance in his work. His researches on these matters earned for him the Fellowship of the Royal Society, to which he was elected at quite an early age. His papers on the early stages of inflammation placed the whole subject on a new and firm basis and remain as classics and remarkable examples of close observation and accurate deduction.

In 1860 the Chair of Surgery in Glasgow became vacant and Lister was appointed to that position, and left Edinburgh to assume his duties as Professor of Surgery. There he had also charge of wards in the infirmary, and it so happened that the wards allotted to him were particularly insanitary, and in fact all forms of septic diseases of wounds were constantly present in them. Indeed, the terrible results which followed injuries and operations were a cause of great distress to him, and he was constantly pondering over their causation and prevention. At that time many surgeons looked on these diseases as inevitable accompaniments of wounds, especially when treated in hospitals, and believed that nothing in the way of local treatment was of any real value in preventing their occurrence. As a result, however, of his reflections on septic diseases and of the scientific work which he had been carrying out, Lister had already, when he took up his work in Glasgow, come very near the solution of the matter.

In his early lectures, in Glasgow, he devoted a great deal of time to the subjects of inflammation and suppuration and their causes, indeed, to such an extent that the students were inclined to think that too much attention was paid to them, but Lister felt that it was the study of these matters which would ultimately give the key to the cause of the troubles which occurred in wounds and might lead to their prevention. He had already arrived at the following conclusions:—Pus is only formed in wounds as the result of irritation of the granulation tissue which covers the raw surface and the formation of granulations must precede suppuration. The most probable cause of this irritation of the granulation tissue is putrefaction of the discharges in the wound. This putrefaction occurs quite early after the injury. The cause of the putrefaction of the blood and serum is something which comes from without and is not an essential occurrence after a wound, because in subcutaneous injuries the tissues are cut or torn across in a similar manner as in open wounds, blood is effused, serum is poured out and yet no suppuration and no decomposition occur; but once the skin is broken, the state of matters is quite different. Lister came to the conclusion that if only he could prevent the putrefaction of the discharges in the wound, no suppuration would occur and the wound would follow the same course as a subcutaneous injury.

Here, however, he came up against a dead wall; what it was that led to this putrefaction was unknown. It was evident that it had something to do with the exposure of the wound to external agencies, and the general opinion at that time was that it was due to the access of the air and especially of the oxygen in the air. But he had long before satisfied himself that it could not be the air itself, or any of the gases that composed it, because in certain subcutaneous injuries, for example in some fractures of the ribs where the lung was punctured, air might be present in the tissues in large quantity and in contact with effused blood and serum, and nevertheless no inflammation or suppuration or decomposition took place. Hence the conclusion he came to was that it was not the air itself, but something which was conveyed by the air, which caused the trouble, and in former times this something was spoken of as a *miasm*. It will thus be seen that he had advanced a long way towards the solution of the problem which was occupying his mind, and that all that was wanting was the discovery of what it was that, carried by the air or present in it, entered the wound from without and set up decomposition and irritation and the various troubles which, in his opinion, resulted therefrom.

During these early years in Glasgow he tried in all sorts of ways to prevent this decomposition of the fluids in the wounds. He paid attention more especially to personal cleanliness, which he carried out to such an extent that his scrupulousness in that direction led to very sarcastic remarks being made about him. At that time surgeons very often did not even wash their hands after handling a suppurating case and before dressing the next patient, and they were equally careless as to the cleansing of the instruments used; indeed nothing but superficial wiping of a knife or other instrument was done before it was used again. Not only were the surgeons careless in this respect, but also the nurses and dressers, so that infection was unwittingly carried from one case to another. One of the early innovations which Lister made on taking up his work in Glasgow was to institute scrupulous cleanliness, and to insist that the hands and instruments should be thoroughly washed before handling a fresh case. His wards were, therefore, provided with basins, water, and piles of towels, a thing quite unusual at that time.

Lister also carried this matter further and tried to diminish the putrefaction in the wounds by washing them thoroughly. For example, in the case of an amputation, the limb would be held up and kettle after kettle of warm water would be poured over the wound to wash away the pus and decomposing material; he also employed various deodorising substances, more especially Condy's fluid. None of these plans, however, led to any noticeable diminution in the frequency of septic diseases, and it became evident that something more was needed. The use of deodorising materials had also been tried by other surgeons, especially in France, but no real improvement had followed, because the attempts had been solely directed against the smell and not against the bacteria which were the cause of it.

This was the stage which Lister had reached when his attention was called to researches which Pasteur had recently made on the supposed spontaneous generation of micro-organisms and on the process of fermentation. The presence of minute living bodies in decomposing fluids had been known for many years and their origin had become a subject of violent debate. One view was that these bodies arose *de novo* in the decomposing materials, in fact that we had here an example of spontaneous generation of life; others, on the contrary, held that there was no such thing as spontaneous generation and that these minute "animalculæ" were merely the descendants of preceding animalculæ which had been carried into the putrescible fluids along with the dust from the air. Experiments had been carried on for more than a century with the view of settling this question, and though evidence was steadily accumulating against the theory of spontaneous generation it was not till Pasteur took up the question that the final blow was given to this view and that the doctrine of *omne vivum ex vivo* was finally established.

That these minute living bodies, or "vibrios," as the larger forms were termed, had anything to do with the putrefactive changes in the organic fluids in which they were constantly present did not for a long time occur to anyone, and the investigations on spontaneous generation were looked on as of purely scientific interest and not of any practical value. For about fifty years before Pasteur took up the matter, the cause of fermentation had also been very extensively investigated by chemists, especially as regards the alcoholic fermentation, and it had ultimately been demonstrated that this fermentation was undoubtedly due to the growth of the yeast cells which were always present in the fermenting fluids. It had also been quite recently suggested that other fermentations in organic fluids, and among them the putrefactive fermentation, were due to the "vibrios" which developed in them; but here the final proof was furnished by Pasteur's researches. But neither Pasteur, nor anyone who preceded him, with the exception of Davaine, had imagined that these bodies had anything to do with the production of disease. Davaine had, indeed, some years previously, noticed that, in the blood of animals which had died of anthrax, large rod-shaped bodies or "vibrios" were constantly present, and he had suggested that they might have something to do with the disease, but the matter had not been carried further.

These researches had not attracted the attention of medical men; and, indeed, it is hardly to be wondered at that a surgeon, who had much to occupy his mind with his surgical and pathological work, did not happen to be familiar with these recondite and apparently useless researches on spontaneous generation; but when Lister read Pasteur's convincing proof, both of the fallacy of the theory of spontaneous generation and of the relation of living bodies which were derived from pre-existing bodies of the same nature to certain fermentations, among others to putrefaction, and his evidence that these bodies were derived from the dust floating in the air and deposited on surrounding objects, he saw at once that he had found the thing that was wanting. Pasteur's researches showed him that this something for which he had been searching must be a micro-organism, similar to those which Pasteur had described, and a very cursory examination of the discharges from the wounds showed him that they were teeming with living organisms of this kind. Lister, therefore, at once concluded that the cause of putrefaction in wounds, and of the various evils which followed that occurrence, was the growth of micro-organisms in the wounds, that they were derived from the dust in the air and from surrounding objects, and that if he could only prevent the access of these organisms in a living state or destroy them after they had entered the wounds, he might possibly prevent a great many of the evils which followed injuries or operation.

At first, however, Lister had no idea of the far-reaching nature of this conclusion. All that he could say at that time was that, in his experience, these infective diseases did not occur in connection with wounds, such as subcutaneous ones, in which putrefaction of the discharge had not occurred, and he hoped that the same would be the case if he prevented putrefaction in his wounds; his whole aim, in the first instance, was, however, to prevent putrefaction. Nothing was known at that time of the nature of the various infective diseases which occurred in wounds, or their relation to bacteria; indeed, the idea that disease could be due to the growth of any such minute parasites as bacteria had hardly been suggested. It is true, as we have just said, that Davaine, some years before, had pointed out the presence of large numbers of rod-shaped bodies, or vibrios, in the blood of animals which died of anthrax, and he had somewhat timidly suggested that possibly the presence of these vibrios was an essential element in the production of the disease, but this suggestion attracted practically no attention, and it was not until some ten years after Lister's work began that proof was furnished that anthrax was due to the growth of these organisms, which were subsequently named "anthrax bacilli." That pyæmia, septicæmia, hospital gangrene, tetanus, erysipelas, diphtheria, and so on, were similarly due to the growth of micro-organisms in the blood and tissues had not occurred to anyone, and, indeed, did not definitely take root in Lister's mind for some time after he commenced his antiseptic treatment. Nevertheless, Lister had arrived at very definite conclusions on this point, as the result of his experience with the new methods of treatment, long before the part which the various micro-organisms played in the production of disease had been demonstrated.

Let us return, however, to the point when Lister, as the result of his study of Pasteur's researches on fermentation and spontaneous generation, acquired the certainty that the cause of putrefaction in wounds was the access of micro-organisms from without. On reviewing the matter, he saw that the problem of preventing the growth of micro-organisms in wounds was a complex one, and that two different conditions had to be dealt with. On the one hand, the organisms might be already present in wounds before they came under his care, *e.g.* in wounds inflicted accidentally, such as compound fractures; in this case the problem was to kill them in the wound after they had entered, or before they had established themselves there. On the other hand, the wound might be inflicted by the surgeon through unbroken skin, and the problem then was to prevent them from entering in a living condition. In both cases there was the further problem of preventing their entrance during the subsequent progress of the case. His first attempts were made with compound fractures, in which the problem was to destroy the bacteria which had already entered and to prevent others getting in afterwards.

In looking for some agent which would destroy the bacteria and thus prevent putrefaction his attention was directed to some striking results which were being obtained in Carlisle about that time in the treatment of sewage. It was stated that as the result of treating sewage with large quantities of German creosote the material was effectually deodorised and further putrefactive changes ceased in it. Lister accordingly procured a specimen of this German creosote, which contained as its essential element carbolic acid, and he waited patiently till he got a suitable case of a compound fracture in which to test his views, and some months elapsed before such a case presented itself. In the meantime he was occupying himself with the examination of wounds and with the subject of spontaneous generation and other cognate matters. Finally, a case of compound fracture was admitted to his wards and in accordance with his instructions he was sent for at once. He took some of this liquefied German creosote, and introduced it into all the recesses of the wound; he also mixed it with the blood in the wound. The wound was then covered with a small piece of lint soaked in the German creosote, and outside that plain lint and towels were placed to soak up the discharge. The result surpassed his expectations; the patient remained well instead of developing temperature and high fever within a few hours and having a long illness, the wound did not swell or become inflamed, there was no pain and no suppuration. The mixture of blood clot and carbolic acid remained in the wound and a crust formed on the surface. After a time Lister began to try to detach this crust, and found that beneath the superficial layer epithelium had spread over the deeper portion of the clot and the greater part of the wound was covered with a layer of epithelium. The antiseptic which he had employed had prevented the occurrence of putrefaction, and the blood and the tissues in the wound had behaved exactly as if they had been covered with skin, in fact, exactly like a subcutaneous wound. No more attractive scientific paper was ever written than his first publication in the 'Lancet' in 1867 on this new method of treating wounds, and one is struck with the acuteness of his observations and with the rapid and sound deductions which Lister drew from everything that he saw.

As the carbolic acid evaporated from this mixture with blood and might thus leave a putrescible substance, his next step was to paint the surface of the clot daily with fresh carbolic acid, and later on he covered the crust with a tin cap so as to retain the carbolic acid. The character and progress of these wounds was something quite new to him and to everyone else, and it is not surprising that as case after case followed the same course he and his staff became most enthusiastic over this revolution in surgery. After a time he proceeded to apply the same principle to operation wounds, and the first case in which he used it was a patient with a large spinal abscess. This was opened, and the pus caught and mixed with carbolic acid to form a paste, which was applied over the wound with a tin cap outside in the manner just described. When he came to dress the wound next day he found to his surprise that no pus was coming from the wound, but only a little clear serum. This was a new experience, and a thing which had not occurred to him before, and he was in a difficulty, because he wished to apply a fresh mass of the carbolic acid paste outside, and he had no pus or blood to make it with. By this time he had found that carbolic acid was soluble in oil, and he therefore sent to the dispensary for a solution of carbolic acid in 20 parts of linseed oil and some whiting, and proceeded to make a paste or putty with which he covered up the wound, covering the putty with a piece of tin. This acted admirably and the abscess healed up without any fever or general disturbance—a result of which he had had no previous experience.

He was now able to get rid of the undiluted acid with its caustic effects, and had at his disposal oily solutions of carbolic acid and the carbolic putty, and with these he proceeded to extend his system, not merely to accidental wounds but to all sorts of operation wounds, and with the most remarkable success. His plan at that time was to cover the skin with carbolic oil before the operation, to soak his instruments in carbolic oil, and to fill up the wound from time to time during the operation with the same oily solution. It is doubtful if any better results can be found even at the present day than those which were obtained at that time by the use of carbolic oil and putty. At the same time the method had great inconveniences; the putty, for example was very apt to crack and crumble, and the carbolic oil obscured the view.

After many experiments, Lister introduced a specially prepared lac plaster as a substitute for the putty. This lac plaster was made of shell-lac and carbolic acid covered with a very thin layer of caoutchouc. This mixture was spread on suitable material and wrapped round the wound; outside this cloths were applied to soak up any serum which might exude. About this time also he obtained purer carbolic acid and was able to make watery solutions, and these were substituted for the oily ones. He was now able to carry out a more thorough disinfection of the skin than had been possible previously, for he fully realised that, apart from the dust in the air, bacteria might grow on the skin and so spread into the wound. From the first the necessity of disinfecting instruments and everything which came in contact with the wound had been fully realised by him, and at this time the disinfection was carried out by immersion for a considerable time (1–2 hours) in 1/20 watery solution of carbolic acid.

While the lac plaster was a great improvement on the carbolic putty it had the disadvantage that it did not absorb the discharge, and the next point to which Lister turned his attention was to obtain some material as a dressing for wounds which would absorb and retain the discharges while at the same time preventing putrefaction in them. He tried oakum and various other materials and ultimately fixed on the fine gauze material which is still used at the present time, and he did a great deal of work with the view of converting it into a suitable dressing. Though the gauze could be disinfected by soaking it for a sufficient length of time in carbolic solution, the discharge in passing through it very soon neutralised or washed out the antiseptic and quickly underwent putrefaction in the gauze. It was necessary, therefore, to store up the carbolic acid in the gauze, so that it should on the one hand yield up enough to prevent the discharges which passed through it from undergoing immediate decomposition and on the other hand retain enough to avoid the necessity of frequent changing of the dressing. He had already found that carbolic acid had a great affinity for resin and that a resinous mixture only parted with its carbolic acid slowly. But gauze impregnated with resin formed a very sticky material which was unsuitable as a dressing. This difficulty was overcome by mixing paraffin with the resin, and gauze impregnated with this mixture was found to answer the purpose very well. For the sake of economy he placed a piece of jaconet, previously sterilised by immersion in 1/20 carbolic lotion, outside the gauze which was applied over and around the wound so as to prevent the discharge passing straight through it opposite the wound, but to compel it to travel over all the gauze before reaching the surface.

At this time he still laid great stress on infection from the air, and in operating he constantly poured carbolic lotion into the wounds so as to destroy any bacteria which might fall into them from the air, and in dressing wounds a stream of carbolic lotion was kept flowing over the incision for the same reason. It may be said here that, contrary to what has been generally supposed, the lotion was never syringed into the wound after it had been once closed, it merely flowed over the surface so as to prevent living dust gaining access. It was soon found that although septic diseases were now absent, such a free use of carbolic lotion not only obscured the view, but caused a good deal of irritation of the wound, as evidenced by the large amount of serous discharge afterwards, and also frequently led to carboluria, though seldom to any serious signs of poisoning. Hence he recognised the necessity of diminishing the amount of carbolic acid which came in contact with the wounds, while at the same time preventing the access of living organisms, and after many experiments, he at length introduced the carbolic spray. The spray producers were worked at first by hand- or foot-bellows, but later by steam, and a very fine cloud of spray containing about 2½ per cent. of carbolic acid surrounded the wound during the operation and at each subsequent dressing. The idea was that the fine particles of the spray coming in contact with the bacteria floating in the atmosphere would kill them before they fell into the wound, while at the same time only a small amount of carbolic acid would come in contact with the wound and so the local irritation by the carbolic acid and the amount of absorption would be very much less than by the former plan.

With the view of preventing irritation of the line of incision by the carbolic acid contained in the discharges, many experiments were made to find some material which could be interposed between the dressing and the line of incision, and which would be impermeable to carbolic acid. Ultimately a satisfactory "protective" was obtained by covering the ordinary oil-silk used for surgical purposes by a layer of copal varnish. The surface of this prepared oil-silk was then painted over with a layer of dextrine and starch, so that when placed in carbolic lotion in order to disinfect it, it should be wetted all over. A narrow strip of this "protective" was laid over the line of incision before the gauze dressing was applied.

While Lister was thus engaged in improving his methods for destroying bacteria and preventing their access in a living state to wounds, he also spent much time in improvements in the treatment of wounds apart from the question of asepsis. The most important of these improvements was the introduction of absorbable ligatures. Up to the time when he commenced his great work the blood vessels were tied with silk or hemp, and as these ligatures had to separate by ulceration and suppuration before the wound could heal, they were left long and hung out of the wound in bundles, so that they could be pulled out when they became loose, usually about the eighth or tenth day after the operation. As the result of the prevention of suppuration, one of the first things observed was that these ligatures did not separate and there was much trouble in getting rid of them; in fact they sometimes had to be cut short and left in the wound.

Lister accordingly turned his attention to this matter and made many experiments in order to see what happened to the ligature in aseptic wounds, and whether it might not be cut short and the wound closed over it. He found that this was the case and that silk ligatures might be cut short and left without causing any trouble. On examining these ligatures after they had been buried in the tissues for some months he found that they were undergoing destruction and that the threads of the silk were being broken up and absorbed by the cells of the body. The process was, however, a very slow one and after more than a year considerable fragments of the silk were still present. He therefore looked for some material which would be suitable for ligatures and which would be more quickly absorbed, and tried various animal substances, ultimately concentrating his attention on catgut. (Although Lister was not aware of it, catgut had been tried as a ligature material many years previously, but discarded as quite unsuitable. At that time the wounds were septic and the catgut was extruded from the wounds just as silk was, and, besides, as the catgut was not hardened, it very quickly swelled up in the tissues and the knot became inefficient.) Lister very soon found that catgut as it came from the makers was quite useless, it swelled up in a few hours, the knot got loose and when applied to a vessel in its continuity the lumen of the vessel opened up again. He therefore made many experiments on the preparation of catgut so that it should remain firm in the tissues, should not be absorbed for some time, and should at the same time be aseptic. His researches on the preparation of a suitable catgut ligature were carried on intermittently for many years.

Another point to which he devoted much attention at this early period was the drainage of wounds. One of the first points which became evident was that as the result of the irritation of the carbolic acid a large amount of serum was poured out during the first two or three days after the operation and this had to be got rid of, otherwise it distended the wound and interfered with the healing. Drainage of the wound was therefore necessary in order to allow this serum to escape. At first he carried this out by introducing a piece of lint and later of gauze into one corner of the wound, but this proved to be inefficient and objectionable in many ways, and later on he resorted to indiarubber tubes. It is interesting to note that the first patient on which he used an indiarubber tube was Her late Majesty Queen Victoria, for whom he was called on to treat a large axillary abscess. I may quote Sir Hector Cameron's description: "In due course it (the abscess) was opened, with all antiseptic precautions, the line of incision in the skin having first been frozen by the use of Richardson's spray apparatus. Up to that time it had been Lister's practice in such cases to introduce a narrow strip of lint dipped in an oily solution of carbolic acid (1 to 4) through the incision, with the object alike of preventing primary union and of acting as a drain. This practice was followed on the present occasion. Next morning he was disappointed to find that little or no drainage had taken place and on withdrawal of the lint, thick pus, similar to the original contents of the abscess, escaped in quantity. Local tenderness and fever still also persisted. The same state of matters was found at one or two subsequent dressings. During a walk in the open air (a favourite practice with him when trying to solve a knotty problem), it occurred to Lister that if he could make use of some aseptic tubular drain, instead of the oiled lint, matters might progress favourably. Accordingly, on retiring to his bedroom that evening, he cut out a piece of the indiarubber tube of the Richardson's spray apparatus of suitable length and, having cut holes in it and sewed into one end of it a piece of silk thread, he placed it to soak all night in some watery solution of carbolic acid (1 to 20). In the morning he was pleased to find that the rubber was in no way weakened or altered in structure and, when changing the dressings, he substituted the tube for the strip of lint. At the next dressing he had, as he said to me, 'the inexpressible joy' of finding that not only had free drainage occurred into the antiseptic dressings, but that the discharge was now very thin and watery. Soon it became entirely serous in character, while it rapidly diminished in quantity. All constitutional disturbance disappeared and very soon the abscess cavity was obliterated and complete healing secured. This was the first occasion on which he ever made use of a rubber drainage tube. On returning to Edinburgh, he repeated the experiment in a case of amputation of the thigh, with the best possible results. He immediately had rubber drainage tubes made by the manufacturers and ever afterwards used them constantly. Similar tubes had been devised and used by Chassaignac early in the century for carrying off accumulations of putrid pus from deep-seated situations; but it is my impression that the idea occurred to Lister quite independently. Whether this be so or not, the use of them, when rendered aseptic, proved a valuable addition to antiseptic treatment."

Attention was also directed to the best methods of approximating the edges of the skin so as to obtain primary union. Previously, when wounds were stitched up, it was done in a very perfunctory manner, and the stitches were generally pulled too tight and caused much irritation. Lister took the greatest care to bring the edges together accurately without pinching the skin under the stitches; these stitches were termed "stitches of co-aptation." When there was a moderate degree of tension two or three stitches of fairly thick silver wire were first inserted so as to relieve the tension (stitches of relaxation) and then the stitches of co-aptation were introduced. When much skin was removed he had a further arrangement of "button stitches," pieces of lead placed on each side of the wound connected by a piece of silver wire passing through the wound from one side to the other and fastened to the lead plates. The silver wire was pulled tight and caused marked approximation of the edges; in these cases the other two forms of stitches were also employed. The materials used for stitches were silk, catgut, horse-hair, silkworm gut, and silver wire, according to the circumstances of the case.

In many operations on the extremities he also took steps to render the limb bloodless before the operation. This was done by elevation of the limb for three or four minutes so as to empty it of blood, and then a tourniquet was applied at the upper part of the limb. At a later period than that of which we are speaking, Esmarch introduced a method of obtaining a bloodless limb by first bandaging it firmly from below upwards with an elastic bandage, and then applying an elastic band just above the termination of the bandage and removing the latter. There are various objections to the use of the bandage, and Lister performed a number of experiments on animals which showed that his method of elevating the limb was quite as satisfactory as the bandage (see Lister's 'Collected Papers,' vol. I, p. 176) and free from objection; he, however, adopted Esmarch's elastic band in place of the tourniquet.

This was the state of matters when the author first began work as dresser in Lister's wards in 1873. We had carbolic lotions, carbolic spray, and carbolic dressings, protective and jaconet; the vessels were tied with catgut, which was cut short, the deeper parts of the wound were in some cases approximated with catgut, and great care was taken to unite the edges accurately without tension. We had also drainage tubes, and in many operations on the extremities the limbs were rendered bloodless. The instruments, sponges, and anything which came in contact with the wound were sterilised by prolonged immersion in 1–20 carbolic lotion, and the surgeon's hands and the skin of the patient in the region of the operation were thoroughly disinfected by carbolic lotion. As a result septic diseases were entirely abolished. There was no pyæmia; erysipelas was hardly ever seen, unless in cases already suffering from it when admitted; there were no cases of hospital gangrene, nor of tetanus developing after operation. It is true that suppuration did occasionally occur in a wound, perhaps three or four cases in the course of a session, and when this took place very thorough investigation was made as to the source of infection, so as to avoid it in future. In this way the treatment was steadily improved, and with experience the necessary precautions became more and more automatic.

When one bears in mind that these advances had been made in the treatment of wounds in some seven and a half years, and that the methods, starting with the use of undiluted impure carbolic, had been constantly improved till they had reached the stage described above, one can realise what a prodigious amount of labour and thought had been expended on it. During all this period improvements in the surgical procedures, as apart from the question of asepsis, were also constantly being carried out in all directions, as will be presently referred to. Further, Lister carried on his duties as Professor of Surgery and Clinical Surgery most efficiently and conscientiously, and also his hospital and private work. When we consider all this it is clear that the results could only have been reached by a man of splendid physique, and gifted with extraordinary mental endowments and powers of observation and deduction.

Although at this time (1873) septic diseases had been abolished and the range of surgical work had been greatly widened, Lister was not satisfied that he had worked out the best possible method and was getting the best results. The model which he had always before him was the subcutaneous wound, and his aim was that as soon as the operation was over and the wound stitched up it should become practically a subcutaneous wound and that only a certain period of rest should be necessary to complete the cure. The perfect result would be that at the end of the operation a dressing would be put on to protect the line of incision, and that when it was removed at the end of a suitable length of time (say, eight to ten days) the wound would be found healed, not only at the surface, but in depth, the stitches would have become absorbed, and nothing further in the way of dressings would be required.

To attain this ideal two things were necessary, firstly, that living bacteria should be completely excluded from the wounds, and, secondly, that the means employed to keep out the bacteria should not unduly injure or irritate the tissue. The first had already been attained almost completely, and there could be no doubt that with further experience the exclusion of infective bacteria could be relied upon. But as regards the second point, the means employed to secure asepsis of the wound did irritate the tissues sufficiently to prevent the realisation of the ideal and led to the exudation of serum to such an extent that drainage was as a rule necessary for two or three days, and so the wound could not be closed and left alone under one dressing. Further, carbolic acid is a poison, and if absorbed into the system in large quantity might give rise to disagreeable results. As a matter of fact symptoms of serious poisoning from absorption of carbolic acid were very rare, but a good many patients had carboluria, which showed that a certain amount of absorption did take place. Further, the method was complicated, and many men would not give the time and care which was required in order to ensure success. The disadvantages of the treatment were, however, not of very much importance in contrast to the fact that septic diseases could be completely avoided by its use, but nevertheless Lister was not the man to rest content with anything short of his ideal, and consequently his further work was in the direction of simplifying the methods and of reducing the irritation of the wounds as far as possible.

With this object Lister examined every fresh substance which was suggested as an antiseptic, testing them bacteriologically and observing their effects on wounds. He was constantly coming to the hospital with mysterious packets containing all sorts of gauzes or ointments, with which suitable wounds (*i.e.* those in which there would not be any serious danger if the asepsis were not quite perfect) were dressed, and the results as regards asepsis and irritation were carefully observed. He generally tested them as regards irritation on his own skin in the first instance, and he was frequently seen wearing patches of dressings on his arms in order to test if they irritated the skin. A great variety of substances were tried in this way, such as boracic acid, salicylic acid, picric acid, eucalyptus oil, thymol, various mercurial preparations (bichloride of mercury, biniodide of mercury, albuminates of mercury), iodine, iodoform, chinosol, and so on; in fact, whenever a new antiseptic substance was suggested it was carefully tested bacteriologically, its effect in preventing putrefaction of blood was examined, its irritating properties and its utility as an application to wounds were tried. The great majority of those substances were rejected for one reason or other, but a certain number were retained and are still employed in surgical practice; such as boric lotion, boric ointment, and boric lint, eucalyptus ointment, lotions of bichloride of mercury, double cyanide of mercury and zinc gauze, salicylic wool, etc.

When the mercurial preparations were introduced the use of carbolic acid was very much restricted, and ultimately it was only employed for the disinfection of the skin, and for keeping the instruments sterile during an operation. Sublimate lotions were substituted for carbolic acid, for the cleansing of the hands and sponges during the progress of an operation, and carbolic dressings were replaced by gauze impregnated with mercurial salts.

In the case of the mercurial dressings it became necessary to disinfect them before use, because, the mercurial salts being non-volatile, bacteria falling on them were not destroyed; this was at first done by wetting them with 1/60 carbolic lotion, and later by heat. The greatest simplification was the abolition of the carbolic spray. As bacteriological science advanced it was shown that the bacteria floating in the air were rarely pathogenic, and, indeed, were unable to grow in wounds and therefore might be disregarded. Further they were present in the air in the spore form, and the carbolic spray was shown to be ineffective in killing spores. When these facts were demonstrated it became evident that the spray was unnecessary and did not fulfil its object, and Lister accordingly gave up its use. No one was more pleased than Lister to get rid of the spray; so long as it was possibly of use he did not dare to discard it, but once it was shown not to be necessary he gladly gave it up.

Lister had always a strong belief in the *vis medicatrix naturæ,* and was always pointing out facts which indicated how the tissues, if in a healthy state, could to a certain extent prevent the growth of bacteria, and actually destroy them. Indeed, he was the first to furnish definite proof that what he termed the "vital action" of the tissues was a very potent agent in protecting the body from bacterial invasion. He showed by experiments with urine and milk how organisms cannot spread up canals lined with healthy mucous membrane. He disinfected the orifice of the urethra and the glans penis, and passed urine into a sterilised flask, which was then plugged with cotton wool. This remained sterile for years, and one flask is still in existence. A similar experiment was performed with milk; the udder and teats of the cow were disinfected and milk drawn into sterilised vessels. This is a much more difficult test, as the air of cowsheds is full of bacteria of all kinds, but several tubes of milk procured in this way remained sterile, showing that bacteria were unable to penetrate up healthy milk ducts.

By the time that Lister gave up work he had simplified and perfected the treatment of wounds to a very great extent. Antiseptics were no longer brought in contact with wounds in any appreciable quantity, and the antiseptics which might possibly get in were much less irritating than those formerly employed. As a result there was much less discharge from the wounds, and drainage was no longer necessary unless in exceptional cases. Consequently, dressings might be left on for days, and when removed the wound was found to be healed, while if easily absorbable catgut stitches were employed, they came away of themselves. In fact, his ideal of converting an open wound into a subcutaneous one had been practically attained. Since that time further changes of a minor character have been made which have added to the security of the aseptic result, such as the use of boiling for disinfecting instruments and other materials, the sterilisation of dressings, gowns and so on, in high-pressure sterilisers, the use of sterilised rubber gloves, masks, etc. Other changes have also been made which,

however, do not always show the profound insight and thoroughness of the Master, but, nevertheless, aim at still more perfectly attaining his ideal.

The principles on which Lister worked were on the one hand to keep living bacteria out of wounds, and on the other to remove as far as possible anything which would hinder nature to carry out its work, viz., the repair of the wounds, as quickly and satisfactorily as possible; in fact, as has already been said, he aimed at converting an open wound into a subcutaneous one. We have sketched the methods by which these aims were gradually attained in the course of a long and most laborious research. What then were the results? They were the complete abolition of suppuration and the septic diseases associated with wounds.

Previous to the introduction of his methods a surgical ward was a most depressing and painful sight. The odour in the wards, in spite of ventilation, was nauseating and often foul, the patients were suffering, in pain, flushed, feverish, and very ill; some were dying of one or other of the septic diseases of wounds. Union by first intention was a very rare occurrence; suppuration occurred in practically every case; indeed it could hardly be otherwise, as in most cases bunches of ligatures were hanging out of the wounds; associated with these ligatures was the danger of secondary hæmorrhage when they began to separate.

Still more serious than these local troubles was the frequent occurrence of general septic diseases, such as septicæmia, pyæmia, erysipelas, tetanus, or hospital gangrene. In a large proportion of the cases in which a wound of any considerable size was produced, whether by accident or by the surgeon's knife, the patient suffered more or less severely from one or other of these surgical diseases. After major amputations, for example, the mortality was very high; the average in the practice of various surgeons at that time varied from 30 to 50 per cent. We may here quote from the introduction to Lister's 'Collected Papers':—

"Lister collected his statistics of amputation for two years (1864 and 1866), just before he introduced the antiseptic method of treatment, and found the mortality to be 45 per cent. The causes of death are not definitely stated, but almost all the deaths were due to infective diseases; for example, of six deaths following amputation of the upper extremity four were due to pyæmia and one to hospital gangrene. In his paper on excision of the wrist-joint, published in 1865, he refers to fifteen cases in which he had performed this operation, and incidentally remarks that six were attacked by hospital gangrene, while one died of pyæmia.

"Volkmann, in one of his earliest papers on antiseptic treatment, stated that for the four years preceding the adoption of Lister's method, that is down to 1872, he had left his wounds entirely open. During the first year in which this method was carried out, the results were very favourable, and he was thoroughly convinced of its superiority over the plans which he had formerly adopted. As time went on, however, and as overcrowding of the wards became unavoidable, infective diseases of wounds increased progressively, and at last, in the summer and autumn of 1871, the deaths from pyæmia and septicæmia were so numerous that he made up his mind to close the hospital altogether for a time. Before resorting to this desperate remedy, however, he determined to try the Listerian method for a few weeks, and the result of this trial was entirely to alter the aspect of affairs.

"Similar facts were published by Nussbaum of Munich, who commenced the treatment two years later than Volkmann. The hospital at Munich, a building by no means satisfactory as regards sanitary arrangements, became a hotbed of septic infection, to so great an extent that almost every case of open wound was attacked by one or other of these diseases. Pyæmia was rife, affecting nearly all cases of compound fracture, wounds of bones, and amputations. Erysipelas was constantly present. During 1872 hospital gangrene also appeared and steadily spread in spite of all the precautions which experience dictated or ingenuity could devise; in that year 26 per cent. of all the wounds were attacked by this dreaded disease; during 1873 the proportion increased to 50 per cent., and it ultimately reached 80 per cent. Erysipelas, too, which in 1872 was of a comparatively mild type, became much more virulent as well as more frequent. All this occurred in spite of the use of antiseptic lotions, of the open method, and other devices. In 1878, after he had put Lister's method to the test of practice, Nussbaum published an essay entitled 'Sonst und Jetzt,' in which he drew the following striking contrast between the previous state of affairs and that which followed the introduction of Listerism:—

Formerly.	*Now.*
Injuries of the head, compound fractures, amputations, and excisions, in fact almost all patients in whom bones were injured, were attacked by pyæmia. For example, of 17 cases of amputation, 11 died from this cause. Even patients with severe whitlow died from it.	No pyæmia.
Hospital gangrene had got the upper hand to such an extent that, in spite of the open method, in spite of continuous water-baths, in spite of the use of chlorine water, or the actual cautery, finally 80 per cent. of all wounds and ulcers were attacked, large arteries being opened into.	No hospital gangrene.
Almost every wound was attacked with erysipelas	No erysipelas.

"It would be easy to produce a great cloud of witnesses to the appalling state of matters in various hospitals before the introduction of the Listerian method, but their testimony would merely be a repetition of the above statements. It is true that these untoward results were witnessed most often, and in their direst form, under hospital conditions of a particularly insanitary kind, and that their frequency and severity varied considerably, according to the methods of wound treatment adopted. Nevertheless, these infective diseases were present everywhere, and it will readily be understood that the dread of them, never absent from the surgeon's mind, was a serious bar to progress."

But the results were much more far-reaching than this. The disappearance of septic diseases after operations opened up a new and large field of surgical activity Prior to the aseptic period, only operations of urgent necessity were performed, and these chiefly consisted of amputations, strangulated hernia, colostomy, a certain number of excisions, operations for stone in the bladder and for diseases of the urethra, opening abscesses, ovariotomy (just in its infancy) and a number of minor operations, especially such as could be performed subcutaneously. When it was demonstrated that one need no longer fear septic diseases after operation, it became evident to Lister that the whole subject of surgical treatment must be reviewed, and that many conditions might be relieved by operation which were formerly left alone, or that operations might be more thoroughly done than had previously been the case, and that many operations might now be performed which had not been thought of, or, if suggested, had been condemned as impossible and even criminal. Into this matter Lister threw himself with enthusiasm, and the treatment of every case was carefully considered from the point of view of whether something better might not be done for it under the protection of asepsis than had hitherto been the case. Lister thus not only abolished septic diseases, but was the pioneer of modern operative surgery, and for years surgical work could be seen in his wards which was not done elsewhere.

Reverting to the period when the writer began to work under him (1873), we had, on the one hand, the Professor of Surgery and several surgeons at the Edinburgh Infirmary who had not adopted Lister's practice, and ridiculed his views, and on the other, Lister himself. The teaching of the two schools was often diametrically opposite, and those of the anti-Listerian school were not sparing in their scathing and sarcastic denunciation of his work. His teaching was, however, clear and logical, and when we (the students) compared the practice and results of the two schools there was no question in the minds of anyone which was right and which was the surgery of the future. It would take too much space, and might be wearisome, to narrate the many alterations which Lister made in surgical practice, but long before his views on the treatment of wounds had been generally accepted, he had entirely altered the operative treatment of the surgical diseases which came under his notice. We may mention, however, a few points.

A good deal of his early advances in practical surgical work was carried out in connection with injuries and diseases of bones and joints. Ununited fractures of bone had previously been occasionally treated by operation with the view of obtaining union, but generally with disastrous results. Lister, however, had no hesitation in carrying out operations of this nature and with great success. Even if the operation involved opening a neighbouring joint, as in fractures of the olecranon, he did not hesitate to perform it. Malunited fractures were also operated on, pieces of bone being removed as required and the ends being brought into proper position and fixed by silver wire. Recent fractures were also treated by operation if the bones could not be got into position. Deformities of bone, such as those which occur in rickets, were corrected by open operation, and old-standing dislocations were reduced by operation, the joint being freely opened and prepared for the reception of dislocated end. Healthy joints were opened for the removal of loose bodies. Joints were drained for obstinate hydrarthrosis. Tuberculous joints were laid freely open and drained. Extensive operations were performed for mammary cancer, the axilla being freely opened up. Attempts to cure hernia by operation were made. He reintroduced suprapubic lithotomy in preference to lateral lithotomy; and, indeed, in many other diseases too numerous to mention, he introduced operative treatment long before others had taken up aseptic work and had rid themselves of the former teaching that such operations were too dangerous. Had he published the work which went on in his wards he would be known, not only as the greatest scientific surgeon and the greatest benefactor of humanity who has ever lived, but also as the greatest practical surgeon of his day.

Although he was often urged to publish his practical work he always refused to do so, the reason being that he knew that there were very few surgeons who had at that time taken up aseptic work, or who were capable of keeping a wound aseptic; indeed, there were very few surgeons who did not look on his theories and practice as a species of insanity. He felt that if he recommended in the medical press procedures, the safety of which entirely depended on the rigid asepsis of the wounds, he might tempt other surgeons, who were not skilled in aseptic work and who did not, indeed, believe either in its theory or its practice, to undertake similar operations which, without the protection afforded by his work, would have ended in the most terrible disasters.

Another direction in which his activities found vent was in bacteriological work, much of which he also did not publish. He constantly made bacteriological observations and experiments in connection with his work on the treatment of wounds, and he very soon saw and taught that there was a good deal more to be considered than the simple putrefaction of the discharges in the wounds. He found, for example, that although there was no putrefaction in the discharge as tested by the sense of smell, there might still be pus, and he promulgated various theories as to the production of pus apart from the growth of micro-organisms. He, however, before long gave up those theories and had to take another view, namely, that the organisms which produced putrefaction were not necessarily the same as those which produced suppuration, and that organisms might produce suppuration without any putrefaction being present in the wound.

As a result of the disappearance of the various septic diseases in his cases, he naturally adopted the view that they must be due to the growth of micro-organisms, not merely in the wound, but in the tissues of the body. He was the first to point out that the tissues of the human body have a powerful action in destroying bacteria and in preventing their spread, and that they were not like dead inert tissues, ready to become the home of organisms if only the latter gained access to them. He further came to the conclusion that these various septic diseases must be due to different forms of organisms, and some years after the commencement of his work, he with great diffidence promulgated the idea that tetanus, which at that time was looked on as a disease of the nervous system, was really nothing more than an infective disease of wounds, a view which was laughed to scorn by his contemporaries. It is remarkable how many of the views which he was constantly expressing to his intimates, and indeed to his students, have been verified by the progress of bacteriological work.

Apart from bacteriological work directly bearing on the best methods of excluding bacteria from wounds, Lister made several bacteriological researches in other directions. He made many experiments on spontaneous generation to test the accuracy of the previous results, and also extensive observations on the germ theory of fermentative changes. At the beginning of his work, he spoke of bacteria in connection with wounds as if they were all the same, but very soon he saw that there must be many different kinds of bacteria, each with their own special effect on the medium in which they grew. He was the first to demonstrate this point in his beautiful research on "Lactic fermentation," published in 1877, in which he showed that this change in milk was due to a particular organism which he called the *Bacillus lactis*, and which he was able to isolate by a very ingenious method from the many other forms of organisms which are present in milk.

In the course of his work on the best antiseptic materials for dressings (which he usually tested by packing them loosely in a tube, saturating them with fresh blood and then inoculating the blood at one end of the tube with putrefying material) he made the remarkable observation that a definite quantity of the putrid material must be inoculated in order to start the putrefactive process in blood, and that if the quantity was too minute, even though it contained a number of bacteria, putrefactive changes might not occur, indeed the organisms would not grow. The writer does not remember if this observation was ever published ; it may have been mentioned in some of his papers, but it led the author to investigate whether anything of the kind occurred in infective diseases in the living body. These researches showed that a similar condition prevails in infective diseases, and that the occurrence and the virulence of the infection in individuals not extremely susceptible to the disease depend to a very large extent on the dose of the infective material in the first instance, a fundamental and very important point in the natural history of these affections.

In addition to his antiseptic and scientific work, Lister wrote several papers on surgical subjects which were characterised by the same care and thought as were his other works. Of these, his articles on "Amputations" and "Anæsthetics" published in Holmes's 'System of Surgery,' and his paper on "Excision of the Wrist," may be specially mentioned. These articles alone would suffice to stamp him as a good practical surgeon.

This short sketch will give some idea of the great work which Lister carried out and which has completely revolutionised surgery. He had the great good fortune to live to see his views universally accepted and the revolution completely accomplished, and those who were associated with him well know the great joy which it was to him to realise the enormous saving of life and diminution of suffering which resulted from his work. But although he had this supreme satisfaction he still remained to the last the modest man which he was at the beginning, always ready to accept anything which seemed likely to improve matters and to listen to and ponder over the views of others. Perhaps he was too ready to accept the work of others, only to find when put to a thorough test that it failed and had to be abandoned; this was especially the case in his search for more satisfactory and less irritating bactericides. More than once he announced to his class that such and such a surgeon had discovered the ideal antiseptic, only to find on thorough examination that it was not so.

Lister's patience and industry were extraordinary, indeed without these qualities he could not have carried out his work. From early morning till late at night he was always at work, patiently testing his conclusions, trying new plans and materials with the view of improving and simplifying his methods, looking after his patients, teaching his students and explaining his views to the visitors who crowded his lectures. As a teacher he was unrivalled. His diction was clear and simple, he gave his reasons fully for everything which he did, his lectures were thoughtful and made his hearers think, and his earnestness was inspiring. He did not teach the students to pass examinations, he taught them to think, he inspired them with high ideals, and those who had the honour and good fortune to work with him worshipped him and humbly set themselves to follow his example.

One of his great characteristics was his conscientiousness. He never acted on impulse, but always carefully considered any step he took. This is very evident in his scientific work, as a study of his writings will show. He made rapid deductions, but he tested them and considered them carefully before they took a place in the edifice which he was building, and he was always open-minded and ready to modify or abandon his positions if they proved to be wrong. In his private life, and in his dealings with patients, his conscientiousness was always in evidence. The advice which he gave was always the result of careful deliberation, and the welfare and comfort of his patients, whether private or hospital, were his first care. He spent a great deal of time in his hospital work, not only in dressing and making observations on the cases, but in seeing that every detail in the treatment was thoroughly carried out and that the patients were made as comfortable as possible. If a patient complained that a bandage was too tight or that his dressing was uncomfortable he would stop and rectify it himself, no matter what other engagements he had made. As a result he was often unpunctual in keeping his engagements. His humanity and his desire to benefit his fellow men, whether medically or financially, were very striking traits of his character. He was ready to believe any tale of distress which was brought to him, and was very frequently imposed upon to a great extent by professional beggars. Indeed it was often difficult to persuade him that the recipients of his charity were unworthy; he had a childlike belief in the veracity and honesty of mankind.

Lister was elected a Fellow of the Society in 1860. He served on the Council in 1881–3, 1893–1901, and 1902–3. He was Foreign Secretary in 1893–5 and President during 1895 to 1900. He was also a Vice-President in 1900–01. In 1880 he received a Royal Medal and in 1902 the Copley Medal.

It is not possible to close this review of Lord Lister's work and life without referring to the great part which Lady Lister took in his work. She was an ideal helpmate—quiet, unassuming, and wrapt up in his work. The notebooks of his experiments are almost entirely in Lady Lister's handwriting, and not only did she act as his assistant, but she aided him in discussing and criticising the results obtained. Her loss to him was irreparable, and he was never the same afterwards. Fortunately her death did not occur till his work was finished.

W. W. C.

GEORGE ROBERT MILNE MURRAY, 1858–1911.

George Robert Milne Murray was born on November 11, 1858, at Arbroath, Forfarshire, Scotland, and after receiving a general education at the High School of that town, proceeded to the University of Strassburg, where he made a special study of Cryptogamic Botany under the direction of Prof. De Bary. On his return to England, in 1876, he was appointed assistant in the Botanical Department of the British Museum, the Botanical and other Natural History Departments being at that time located at Bloomsbury; he was placed by the Keeper, Dr. W. Carruthers, F.R.S., in charge of the Cellular Cryptogams.

While Murray had charge of the Fungi and Algæ, and more especially after the removal of the botanical collections from Bloomsbury to South Kensington, the cryptogamic herbarium was not only rearranged on modern lines, but largely extended through the acquisition of various important collections for the Nation. In this connection may be mentioned Broome's valuable herbarium of Fungi, Dickie's marine Algæ, and Wheeler's extensive series of water-colour drawings of British Fungi. He realised fully the importance of authentic microscopic preparations to the student of Cryptogams, and was instrumental in acquiring several valuable series of mounted slides, for instance, those illustrative of the Fungi and Plant Anatomy of De Bary, the Red Algæ of Schmitz, and the Diatom collections of Deby and of Comber. His first published note dealt with the reproduction of the Ascomycetes and appeared in 1877. He contributed to the 'Encyclopædia Britannica' an article on Fungi in 1879, and one on Vegetable Parasitism in 1885. In 1882 Murray was asked by Prof. Huxley to investigate the Salmon disease, and afterwards published three reports on that important subject. He was lecturer on Botany at St. George's Hospital Medical School for four years (1885–9) and afterwards at the Royal Veterinary College (1890–5). In 1889 Murray (in conjunction with A. W. Bennett) published a Handbook of Cryptogamic Botany. From 1891, onwards, he was secretary to the West India Islands Exploration Committee—a Joint Committee of the Royal Society and the British Association—which was instrumental in the collection of much valuable material, comprising more especially Cryptogamic Plants and insects.

In 1892 he initiated and edited 'Phycological Memoirs, being Researches made in the Botanical Department of the British Museum,' and several parts were published in that and succeeding years. In 1895, on the retirement of Dr. Carruthers, he was appointed Keeper of the Botanical Department of the British Museum, and two years later elected a Fellow of the Royal Society.

Murray was born within a stone's throw of the sea and was at his best and happiest on that element. Hence he was led to make a special study of the marine side of Botany, publishing an Introduction to the Study of Seaweeds in 1895. As a collector of the minute vegetable organisms present in sea-water he was indefatigable, and in 1897, for the special purpose of collecting plankton, he obtained a grant from the Royal Society and crossed the Atlantic to the West Indies and Central America, accompanied by his Museum colleague Mr. V. H. Blackman, now Professor at the Imperial College of Science and Technology; one important result of this expedition was the observation of the hitherto mysterious organism Coccosphæra in the living state and the discovery of its green algal-like nature. This voyage yielded also a rich harvest of new forms of Peridiniæ.

He had previously visited the West Indies in 1886 as naturalist attached to a Solar Eclipse Expedition. From the Scotch Fishery Board steam yacht, the "Garland," he collected diatoms in most of the Scotch lochs; from material obtained in this way he made a very important discovery, that of the reproduction of a diatom by asexual spore-formation. Not only was he an enthusiastic collector himself, but his enthusiasm was of so infectious a nature that he was able to persuade several captains of ocean-going steamers to learn the methods of collecting plankton, and to bring him material from the Atlantic, Pacific, Arctic and Indian Oceans, and from the Red and China Seas. In 1898, aided by grants from the Royal Geographical Society, the Drapers' Company and the Fishmongers' Company, he chartered a tug (the

"Oceana") and went in the month of November to a part of the Atlantic, 300 miles west of the Tearaght Light, off Dingle Bay, Ireland, where the depth quickly increases from 500 to 1800 fathoms, to collect material at numerous measured intervals between the surface and the ocean-bed; on this expedition he was accompanied by V. H. Blackman, J. W. Gregory, L. Fletcher, his Museum colleagues, and also by J. E. S. Moore and Louis Sambon. That part of the Atlantic is so far from the usual lines of traffic that no other vessel was sighted during a space of ten days. Just as the work was being finished, a storm came on of such violence that a train on the nearest land was blown off the rails; the captain allowed the tug to run before the gale and eventually made for Queenstown, where the vessel was detained on account of the storm for thirty-six hours before the return voyage could be continued.

In 1901 Murray, as Scientific Director of the "Discovery" National Antarctic Expedition (under Captain Scott), was extremely busy with the provision of stores and apparatus; further, he edited the 'Antarctic Manual,' which was prepared for the use of the staff and published; he sailed with the "Discovery" from Gravesend and continued the organisation of the scientific work until the arrival at Cape Town, farther than which his duties in London would not allow him to travel. For a year or two previously he had been in unsatisfactory health as a result of repeated attacks of influenza, and it had been hoped that the sea-voyage would act as a restorative, but the heavy strain of the work he had undertaken proved too much for his weakened constitution. Then came two heavy blows: his wife died suddenly, from heart failure, in 1902, and his only brother, Alexander Stuart Murray, after a very short illness (pneumonia), in 1904; his brother had been Keeper of Greek and Roman Antiquities in the British Museum since 1886. Murray's health then broke down completely, and in 1905 he retired to Stonehaven, Kincardineshire, where he passed the brief remainder of his life; the immediate cause of death, which took place in his fifty-fourth year, on December 16, 1911, was cancer in the throat. He has left a son and a daughter. Murray was an excellent and cheery companion, very kind-hearted, and always ready with sympathy and help for those who needed them.

L. F.
W. C.

ADAM SEDGWICK, 1854–1913.

Adam Sedgwick was born in 1854 at Norwich, where his parents were temporarily residing. His father, the Rev. Richard Sedgwick, was vicar of Dent in Yorkshire, and it was there that Adam's childhood was passed, and he always regarded himself as a North-countryman. His father's family had been connected with the neighbourhood for many generations; they were landowners of the kind locally known as "statesmen," *i.e.* they farmed the land which they owned. To this family belonged also Adam Sedgwick, Professor of Geology in the University of Cambridge, and great-uncle of the subject of this sketch. The older Adam was one of the founders of British geological science, and his name is immortalised in the Sedgwick Memorial Museum of Geology at Cambridge. The mother of the younger Adam also belonged to a land-owning family whose seat was in the neighbouring county of Lancashire, and there is no doubt that Sedgwick owed many of the sterling elements in his character to this double strain of country-bred ancestry. Though his studies as a scientific man led him to radical views on many subjects, those who knew him best were never in any doubt as to the existence of an underlying stratum of conservativism on social, religious, and political matters, which formed, so to speak, the bed-rock of his mind.

Sedgwick was educated at Marlborough and entered King's College, London, with a view of qualifying himself for the medical profession, but his stay there was brief, and in 1874 he came up to Cambridge and entered Trinity College as a pensioner. At that time there were being laid at Cambridge the foundations of that school of animal biology which has brought so much fame to the University, and the founders were connected with Trinity College. Michael Foster had left Huxley a few years before in order to introduce the new science of "biology" into the old University; he was Prælector of Physiology and Fellow of Trinity College. Amongst his first pupils was Francis Balfour, later created Fellow and Lecturer of Trinity College, who threw himself with enthusiasm into the study of Comparative Embryology, in which he achieved a world-wide fame. Adam Sedgwick early fell under the spell of this brilliant genius, and soon abandoned the idea of entering the profession of medicine, but took up instead the precarious occupation of a teacher of pure science. In 1877 he took his degree in science with first-class honours. In 1878 he was made Foundation Scholar of Trinity College and he became demonstrator to Balfour.

In 1882 the University, fearful of losing Balfour, created a special Chair of Animal Embryology for him; but, in the same year, Balfour lost his life on a mountain-climbing expedition in the Alps and the University declined to continue his Chair. It seemed as if the newly-created School of Comparative Embryology was doomed to extinction, but after some delay the

University consented to create a Readership in Animal Morphology at the small salary of £100 a year, and to this Adam Sedgwick was appointed. He had already been elected Fellow of his College in 1880 and he was now created Lecturer, and so he was enabled to earn an income sufficient to support himself. It is greatly to the credit of the Fellows of Trinity College that, by their action at this juncture, they endowed biological science and enabled the work of Balfour to be carried on.

The Chair of Zoology was occupied at this time by Alfred Newton, who represented the systematic side of the science, and so for twenty-five years, until Newton's death in 1907, Sedgwick acted as Professor of Morphology and Embryology without either the emoluments or the University status of a Professor. The work begun in Balfour's little laboratory (originally a couple of rooms in the department of Physiology) grew in importance, and room was created for it by raising the roof of the engineering laboratory by means of jack-screws, and thus intercalating a new series of rooms between the roof and what had previously been the uppermost storey of the building. In the lofts thus improvised, Adam Sedgwick, by his perseverance and enthusiasm, built up one of the finest schools of zoological research in the world. The teaching of animal biology, especially on its practical side, was systematised by him to a degree that had never been attempted before, and was converted into a thoroughly sound intellectual discipline. Around him were gathered an eager band of researchers, amongst whom were students from the United States and from Japan. Indeed, the School of Zoology at Tokio may be said to be the child of the school at Cambridge, for Mitsukuri, its founder, began his researches in Cambridge. The pupils of the Cambridge school went all over the country to occupy important positions in zoology; amongst them may be mentioned Weldon, late Professor of Zoology in Oxford; Bateson, Director of the John Innes Horticultural Research Institution; Graham Kerr, Professor of Zoology in the University of Glasgow; Hickson, Professor of Zoology in Manchester. Sedgwick literally lived in the laboratory, bringing the influence of his splendid personality to bear on all his pupils, kindling their enthusiasm, guiding and supervising their researches, and making a most enduring impression on their minds.

In 1883 Sedgwick made a voyage to the Cape, where he remained some months in order to study the embryology of that strange animal Peripatus, which by some naturalists was (and is to this day) classed as an Annelid, whilst others regarded it as the lowliest member of the group Arthropoda. The fruits of this expedition were a remarkable series of memoirs published in the 'Quarterly Journal of Microscopical Science,' which demonstrated beyond cavil that Peripatus is indeed an Arthropod, but one of such primitive character that it might also be regarded as the veritable "missing link" between Annelida and Arthropoda. The relation to one another of blood-spaces and body-cavity were made clear once for all, and a whole mist of misinterpretation cleared away from the ideas which had previously prevailed on the structure and relationships of the Arthropoda. Whilst the publication of his results was still far from complete Sedgwick was elected Fellow of the Royal Society, and subsequently he had twice the honour of serving on the Council. In fact, he was regarded as one of the leading zoologists of the country, and he served on every important Committee connected with the subject. Amongst his staunch and life-long friends he numbered the doyen of British zoology, Sir E. Ray Lankester; for whom he had the greatest respect and admiration. He lived also in the most friendly and harmonious relations with Newton, Professor of Zoology in Cambridge, who, instead of regarding the development of the newer side of zoology with suspicion or jealousy, aided and abetted Sedgwick in every possible way.

In 1892 Sedgwick married Miss Laura Robinson, daughter of Captain Robinson, of Armagh, and in 1897, with some misgiving which was shared by his warmest friends, he accepted the post of Tutor of Trinity College, a position which he held until 1907. The duties of his new office not only rendered it impossible for him to prosecute research, but even made it difficult for him to spend much time in the laboratory. Although he devoted his vacations to the production of a 'Text Book of Zoology,' of which three volumes were published, and which is the most complete so far produced in the English language, yet this was a poor consolation for the withdrawal of his stimulating presence and influence from his students. A comprehensive text book of Zoology is too vast a work to be completed by one man, and although the great interest of Sedgwick's work was the decision of his matured judgment on the infinitely varied facts of the science, yet for the completion of his third volume he had to call to his assistance the help of his friends J. J. Lister and A. E. Shipley (now Master of Christ's College).

In 1907 Newton died and Sedgwick was appointed Professor of Zoology in his stead, but he had hardly settled down to the duties of his new office when events took place which were destined to transfer him to a new sphere of activity. In 1908, the Governors of the newly constituted Imperial College of Science and Technology resolved to re-endow and equip the Department of Zoology in the Royal College of Science, which was one of the three constituent colleges of the new institution. This department had been rather neglected since the death of Howes, who was Huxley's successor; arrangements had indeed been made for the carrying on of the teaching temporarily, but no Professor of Zoology had been appointed. Sedgwick was asked to join the Committee whose duty it was to select a new professor of zoology for the college. When the Committee presented their report, the Governors unanimously besought Sedgwick to accept the post himself and to undertake the duty of reorganising the department. Sedgwick promptly declined; but a short time afterwards the request was renewed, the Rector of the College travelling down to Cambridge in order to press the wish of the Governing Board on him. At last, impressed by a sense of the duty which he owed to the science of zoology in general, he yielded to the pressure put on him and, in 1909, he severed his life-long connection with the University of Cambridge and took up the duties of Professor of Zoology in the Imperial College of Science and Technology. He was succeeded in the Cambridge Chair by one of his ablest pupils, Stanley Gardiner. Once established in London, he threw himself with the utmost zeal into the organisation of the Department of Zoology. He established series of lectures in those branches of zoology which had the most direct bearing on the economic applications of the science. In this way more students were attracted to the department than had ever been in it before, and when Sedgwick died a flourishing sub-department of Entomology, with an able professor at its head, had been brought into existence. The initial task of organisation necessarily involved a considerable expenditure, but Sedgwick's efforts were bearing fruit when sad signs became evident of the failure of his health.

Already, in 1904, he had had some attacks of pulmonary disease, but these, it was thought, had been, completely overcome. There was left, however, some pulmonary weakness, and before accepting the professorship in London, Sedgwick consulted his medical adviser and was assured that he had no disease and was quite competent physically to undertake the duties of the new post. Until the spring of 1911 his health seemed indeed to be improved, and his friends were delighted by his vigour and spirits. In 1911, however, whilst travelling on business in the North of England, he contracted a severe attack of influenza, and from that date until his death his pulmonary troubles increased ; he lost flesh, and his health visibly failed. At the end of 1912, yielding to the urgent advice of his physician and his friends, he obtained leave of absence from his teaching duties and went abroad to winter in the Canaries. The change seemed at first to afford some relief, but a relapse supervened, and, despairing of any improvement in his health, he returned to London, and died in his residence at South Kensington on February 27, 1913, thirty-six hours after his return.

Besides a widow he left three children, two sons and a daughter. The elder son has already entered on an academic career and is a Foundation Scholar in Trinity, his father's college.

In reviewing Sedgwick's contributions to science, his eager and ardent nature must be constantly borne in mind. He was for ever seeking new points of view and was apt to be somewhat impatient of those who clung to older views. Hence he was wont to state his ideas in somewhat strong language, which roused opposition and delayed recognition of the side of the truth which he was seeking to emphasize. But as old controversies pass into oblivion the essential truth of many of Sedgwick's ideas comes more and more into prominence. His early work was concerned with the structure and development of the vertebrate kidney, especially the kidney of the higher vertebrates. In this he was following up and completing the researches of his teacher Balfour. Sedgwick's views were not accepted by Continental zoologists and the great authority of Gegenbaur caused them to be regarded as unsound, but a few years ago their truth was completely vindicated by the masterly researches of Schreiner. It was the same with his work on the kidney of Mollusca: here again his statements were confirmed after a lapse of many years by Plate.

His great work on Peripatus, of fundamental importance for structural zoology, also encountered mistrust on the Continent, but it is not too much to say that all subsequent work, not merely on Peripatus but on the development of Arthropoda generally, has tended to support and confirm Sedgwick's views. Out of this work arose, however, a wider controversy, no less than the validity of the cell-theory itself, and on this subject Sedgwick took up a position which can only be described as revolutionary. The orthodox view of the subject which was then held, and which is still held by many zoologists, is that the higher animal is a cell-republic and that each cell is equivalent to a Protozoon. To this view Sedgwick opposed the idea that the cell of the higher organism is a mere unimportant and imperfect sub-division of the cytoplasm and that a nucleated sheet of protoplasm can and often does replace a layer of cells. Sedgwick's views were received with almost universal repudiation, but the work of Driesch in developmental mechanics has certainly gone far to confirm them; it has been definitely shown that an entire and perfect organism can be built up out of half an egg and that such an organism contains half the normal number of cells, so that each cell in the smaller organism must bear a totally different relation to the whole from what it does in the organism of normal size.

At the beginning of his career Sedgwick was a strong supporter of the recapitulatory theory of development. As the presence of other factors than the ancestral one in the determination of development became more and more obvious, Sedgwick put forward the only plausible theory that has as yet been advanced to explain the existence of the recapitulatory element in developmental history. Later in his life, disgusted with the facile and unsupported assumptions which characterised too much of the reasoning on this subject, he passed into an attitude of entire hostility to the theory and published a series of trenchant criticisms of it in the Darwin Memorial Volume which was issued in 1909. Those who still hold firmly to the truth of the recapitulatory theory can only rejoice in the publication of these able criticisms, for they will compel the data on which the idea of recapitulation is based to be thoroughly sifted, and it is to be hoped that closely considered reasoning will be substituted for the wild guesses which have too often done duty for argument when recapitulation has been discussed.

In appearance Sedgwick might be described as a typical English squire—of medium height, ruddy complexion, somewhat thick-set in build, direct and sincere in his manner. As one got to know him better, his sterling qualities evoked such admiration from his pupils that their feeling towards their master can only be described as the warmest affection. A friend of the most constant and unswerving loyalty, and a friend who would bluntly tell the truth even when the truth was painful to youthful vanity; a wise counsellor, the moderation and carefulness of whose advice were in sharp contrast to the exaggerated language in which he "blew off steam" when off duty: such was Sedgwick, and to all who knew him and loved him his death is an irreparable loss. His very weaknesses endeared him to his pupils when they came to be regarded as symbols of his personality. He had a hasty temper which used to relieve itself in somewhat violent language with a plentiful admixture of expletives—but the storm was over almost as soon as it arose, and the expletives left no sting behind; indeed, they were gleefully repeated and with embellishments served as the basis of a number of Cambridge legends.

Nothing caused more secret gnawing apprehension to his closest friends than the disappearance of these ebullitions; they felt as if the old Sedgwick were slipping away from them, and this proved only too true.

Sedgwick's memory will live for ever in the zoological school at Cambridge, which, though founded by the genius of Balfour, was built up to its leading position by Sedgwick's efforts. Whether the school he founded in the Imperial College will be equally successful remains to be seen, but his memory and example will serve as an inspiration to all those who were brought into contact with him there.

E. W. M.

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